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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 1

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 32, No. 1, January, 1921

ON RELATIONS OF SYMMETRY IN TRANSPLANTED LIMBS

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ONE HUNDRED AND THIRTY-SIX FIGURES

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INTRODUCTION

The circumstance that originally suggested the present study was the apparent difference in the results obtained by Streeter ('07) and by Spemann ('10) in their respective experiments with the amphibian ear vesicle. According to the original account of Streeter, the otocyst, when taken out of an embryo just after closure and replaced after having been rotated 180° on any of its axes, develops in normal posture, though a right vesicle placed on the left side remains true to its side of origin. According to Spemann, the inverted vesicle develops in inverted position, the rudiments of the constituent parts being localized at the time of operation. Although subsequent work by Streeter ('14) seems to have shown that the normal development of the inverted vesicles, found in his cases, was due to their regaining normal posture by rotation as a whole, the original divergence of results nevertheless had raised theoretical questions of great interest, which Spemann has ably discussed. The main question was whether we might have in the otic vesicle an 'harmonic equipotential system' with its future asymmetry in some way stamped upon its intimate structure. Though Spemann's analysis answers the question in the negative, as far as the closed ear vesicle is concerned, it is nevertheless important to determine how far, if at all, systems of this kind are present in the embryo, for their study would throw light upon the question of the mode of representation of adult form characters in the germ, giving evidence from a new quarter with regard to the great problems of development which have usually been approached by way of experiments upon the unsegmented egg and the early stages of cleavage. The method of embryonic transplantation obviously

affords a means of studying this question in any organ or part that in the adult lacks a plane of symmetry. It was with this purpose in view that the present experiments with the limbs of Amblystoma were begun. Limb buds were implanted in both normal and abnormal location, oriented in various ways with respect to the main axes of the embryo-host, and the form and posture of the resulting limbs were studied.

It became evident, after the first experiments were made, that the rudiment of the fore limb behaved differently from the auditory vesicle, no matter whether Streeter's original interpretation or Spemann's was accepted as correct. While it was found that a certain tendency did exist for inverted limb buds to rotate back to normal posture during development, this was not the usual result, nor did the rotation take place in the sense meant by Streeter in his later publication ('14). Furthermore, many irregularities of development were produced by the operations, due largely to the power of the limb rudiment to duplicate itself by budding.[1] On the other hand, it often occurred that buds transplanted from one side of the body to the other developed in harmony with their new surroundings, a right limb bud, for example, placed on the left side, giving rise to a normal left limb.

The earlier experiments which were made in 1911 and 1912 led to no satisfactory general conclusion, so that publication of the work was deferred pending further investigation. Subsequently numerous additional experiments were made, in which more effective precautions against regeneration of the limbs from the host were taken.[2] The situation began to clear when in some of the cases in which the asymmetry of the limb was reversed by

[1] Cf. Barfurth ('94), who showed that supernumerary limbs could be produced in amphibians by regeneration after irregular amputation; Tornier ('05), who obtained multiple appendages by cutting into the limb bud of tadpoles; Braus ('04, '05, and '09) and Harrison ('07), who found that transplanted limb buds frequently give rise to double limbs.

[2] The first experiments were reported to the National Academy of Sciences in November, 1912, at the New Haven meeting. Later reports were made before the American Association of Anatomists in December, 1915 ('16), and before the American Society of Zoologists in the following year ('17 a). The main results have been stated somewhat more fully in the Proceedings of the National Academy ('15 and '17 b).

transplantation it was observed that the reversal came about by a process of reduplication or twinning. By following closely the history of individual cases, it became evident that the double formation was not infrequently obscured by the preponderance of the reduplicating limb bud over the original, so that the former grew into a member of opposite asymmetry, while the original bud was reduced to a mere spur or nodule, which might readily be overlooked. The tendency to produce duplicities thus proved to be even greater than the actual number of fully developed cases indicated. In other cases the reversal appeared to be more direct; at least, a limb of the side of origin often failed to appear as such on the surface, though slight irregularities in the early stages of development, coupled with an appreciable delay in the process, showed that some internal readjustment of the grafted tissue was taking place.

It seemed that the functional activity of the limb, when transplanted to its normal environment, might accentuate the apparently anomalous results just described. In order to eliminate this factor, a series of experiments in which the limb bud was grafted on some other part of the body was undertaken. Here the proper nervous connections did not become established, functional activity was usually lacking or was at best but slightly developed, and the undisturbed effect, upon development, of the relative orientation of the tissues of graft and host could be observed. The latter experiments led to the formulation of the three following simple rules,[3] which hold for implantations either in normal or in abnormal location:

Rule 1. A bud that is not inverted (dorsodorsal) gives rise to a limb of the side of origin of the bud, whether implanted on the same or on the opposite side of the body.

Rule 2. An inverted bud (dorsoventral) gives rise to a limb of reversed asymmetry, whether implanted on the same or on the opposite side of the body.

Rule 3. When double limbs arise, the original one (the one first to begin its development) has its asymmetry fixed in accord-

[3] These rules were phrased somewhat differently in two preliminary communications ('17 a and '17 b).

ance with rule 1 or 2, while the other is the mirror image of the first.

Experiments previously reported[4] have shown that the limb bud is an 'harmonic equipotential system,'[5] and additional experiments with inverted buds (p. 87) and with half buds (p. 83) confirm this result. We must assume, then, that the potencies of the cells of the limb bud to form the fore limb are in the last instance represented in their intimate structure and not merely in their arrangement. The above rules show, however, that not all essential features are stamped upon the constituent elements of the rudiment at the time of transplantation. For example, the difference between the right bud and the left is not an absolute one, since a right limb bud upside down behaves like a left one right side up and vice versa. From this the conclusion has been drawn that the elements making up the limb bud are differentiated in an anteroposterior direction, i.e., along the anteroposterior axis, but are not yet differentiated, at least not irreversibly, along the dorsoventral axis at the period of development at which the transplantations are made. In this one respect the differentiation of the limb is dependent upon its orientation with reference to the dorsoventral axis of the embryo; otherwise as regards its specific form, the limb bud constitutes a self-differentiating system.

These questions will be considered more fully in the concluding section (p. 85).

METHODS AND TERMINOLOGY

All experiments were made upon embryos of Amblystoma punctatum in stages that have been previously defined.[6]

In performing the operations the embryo which is to receive the implanted limb bud is first made ready. If the bud is to be placed in normal location, the wound is prepared as in the extirpation experiments referred to above. A circular incision, hav-

[4] Harrison, '18.

[5] "Jedes kann Jedes und alles Einzelne steht in Harmonie zu einander." (Driesch, '02, p. 229.) See also: '99, p. 72; '05, p. 679, and '08 b, p. 120.

[6] Harrison, '15 and '18.

ing the diameter of three and a half somites (ca. 0.9 mm.) and ventral to the third, fourth, fifth, and half of the sixth myotome, is made, and the disc of tissue, including both mesoderm and ectoderm, is lifted, after which the remaining mesoderm cells are carefully cleaned off. This may all be done without injury to the pronephros, which lies immediately dorsal to the limb rudiment, though if this organ is injured or even extirpated, there is no noticeable effect on the subsequent development of the limb. The embryo thus prepared is held in readiness for the grafting, being secured in position by pieces of silver wire or glass rod bent into proper shape. The limb bud which is to be transplanted is removed from another embryo, as described above, care being taken in lifting it from its bed to take all of the mesoderm possible. It is then transferred on the point of the scissors to the first embryo and fitted into the wound. The orientation of the bud is important and may be carried out as desired by noting the position of pigment markings in the ectoderm of the graft. After it has been properly placed, it is held in position for an hour or more by a single piece of glass rod, bent into such shape that it straddles the embryo, exerting a light pressure upon the grafted tissue. The healing of the wound takes place readily, though frequently a small area of underlying yolk may be left exposed on the border of the wound. This usually heals in a day or two and does not seem to influence the result of the experiment, unless the yolk begins to disintegrate, in which case death of the embryo usually follows.

At the time when the first experiments were made, the conditions necessary to prevent regeneration had not been determined, so that in a number of cases the extirpated area was too small or the wound bed was insufficiently cleaned of scattered mesoderm cells for the result to be conclusive. These experiments have not been included in the tabulations, but will be referred to separately in so far as they are of special interest. In all of the later experiments the size and character of the wound in the host was such as to preclude regeneration of the limb from that source; the resulting limbs must, therefore, have arisen from the engrafted tissue. Even in the cases where the wound was

not especially cleaned, there is no evidence, aside from certain exceptional cases, that the tissue of the wound bed displaces the transplanted bud, though the possibility of its participation in the make-up of the limb cannot be excluded, and it probably actually does take place to some extent.

In transplanting the limb bud to a location other than the normal, the recipient embryo is first prepared as in the other experiments. A wound of proper size is made, usually in the flank just below the ventral border of the myotomes, and the bud grafted in the same manner as described above. In doing this it is well to avoid injury to the pronephric duct (p. 15).

Three different factors regarding the placement of the limbs have been considered in these experiments, viz.: 1) location of the graft in the embryo; 2) the side of the body on which it is placed (whether the same from which it was taken or the opposite); and 3) orientation with respect to the cardinal points of the embryo. The experiments thus fall into eight categories, as follows (fig. 1):

A. Limb buds placed in abnormal location—heterotopic transplantation.

1. On the same side of body as origin—homopleural (*hom.*).

a. Dorsal border of limb bud dorsal with respect to embryo—dorsodorsal (*dd.*).

b. Dorsal border of limb bud ventral with respect to embryo—dorsoventral (*dv.*).

2. On side of body opposite to origin—heteropleural (*het.*).

a. Dorsal border of limb bud dorsal with respect to embryo—dorsodorsal (*dd.*), in which case the anteroposterior axis is reversed.

b. Dorsal border of limb bud ventral with respect to embryo—dorsoventral (*dv.*), in which case the anterior and posterior points of the graft correspond, respectively, to those of the embryo.

B. Limb bud placed in natural location—orthotopic transplantation.

The several categories under this head as under A.

According to the rules stated on page 4, two of the combinations (homopleural dorsodorsal and heteropeural dorsoventral) yield limbs which are of the same side of the body as that on

which they are placed (fig. 2), for the non-inverted limb bud from the same side (*hom.dd.*) does not have its prospective asymmetry changed while the inverted limb bud from the opposite side (*het.dv.*) does. The limbs which develop in these combina-

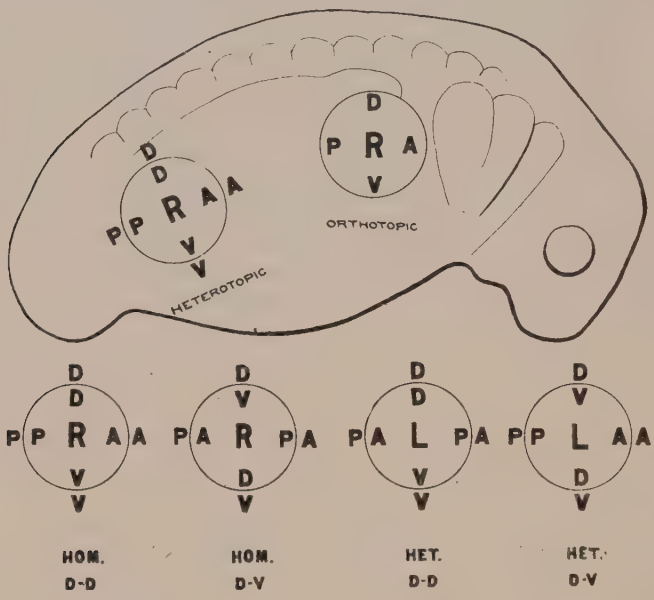


Fig. 1 Diagram showing the eight different operations. The outline of an Amblystoma embryo in the operating stage is shown above. The circles within it represent the limb bud, in the normal (orthotopic) and the abnormal (heterotopic) location. The four circles below represent the four different ways in which limb buds may be oriented with reference to the cardinal points of the embryo; the letters (*D*, dorsal; *V*, ventral; *A*, anterior, and *P*, posterior) within the circles designate the original cardinal points of the transplanted limb, those outside the corresponding points of the embryo. The operations are represented to be on the right side. *R*, right limb bud; *L*, left limb bud; *hom.*, homopleural; *het.*, heteropleural.

tions thus fit in with their surroundings; they have therefore been called harmonic. The other two combinations (homopleural dorsoventral and heteropleural dorsodorsal) give rise to limbs of the side opposite to that of their seat of implantation, for the inverted bud from the same side (*hom.dv.*) has its asymmetry reversed, while the non-inverted bud from the opposite side

(*het.dd.*) remains as it was. The limbs which develop here are not primarily in harmony with their surroundings, so that these combinations have been termed disharmonic.

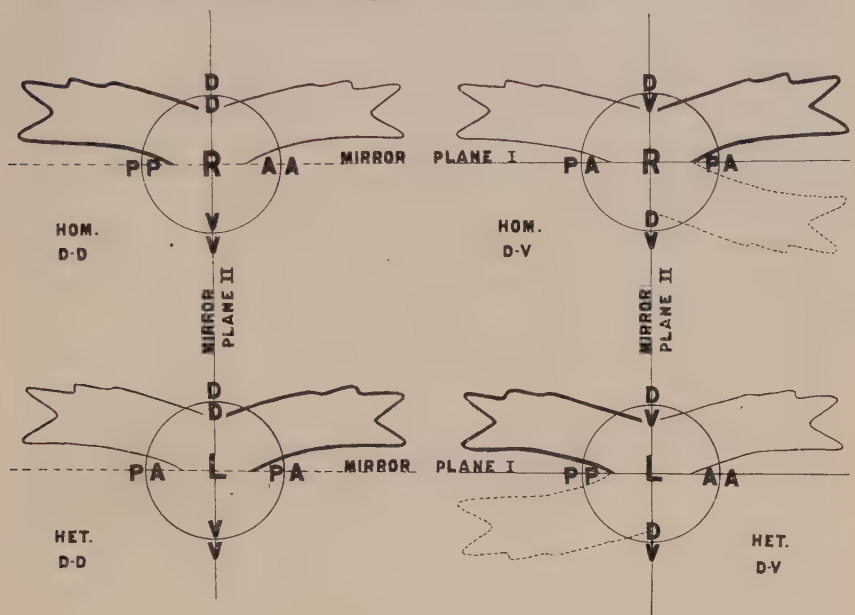


Fig. 2 Diagram showing the results of the four operations, heterotopic or orthotopic, represented as on the right side of the embryo. The circles indicate the transplanted limb buds, the letters having the same significance as in figure 1. Thus the two upper figures in the diagram represent homopleural, and the two lower ones heteropleural transplantations. The two on the left show the transplanted bud in upright (dorsodorsal) orientation while the two on the left are inverted(dorsoventral). The limbs which develop are shown in profile, the ulnar border being uppermost (dorsal) in all which actually develop. A heavy outline indicates the primary member, a light outline the reduplicating one. It is to be noted, however, that the latter develop in by no means all cases, while the former may be resorbed in the heteropleural dorsoventral combination, leaving only the reduplicating member present. The broken outlines show the posture that the limb would have assumed,had it developed as a self-differentiating member totally independent of the influence of its surroundings.

The transplanted limb bud is a flattened disc of tissue, and it is theoretically possible to make eight further combinations by placing the medial surface of the graft corresponding to the lateral surface of the embryo. This is impracticable, however, because

the mesoderm would thus be brought to the surface and the ectoderm buried beneath it. The same effect might be obtained, however, by transplanting the mesoderm alone. While the difficulties in this procedure are great, they have now been in a large measure overcome. The positive experiments are too few in number to warrant any very definite statement, but they do indicate that it is immaterial which surface of the mesodermal disc faces laterally.

A much greater variety of experiments could be had by experimenting with positions intermediate between the upright and inverted positions, i.e., with limb buds turned, say, 90° instead of 180°. Such experiments may yield very interesting results, but as yet there has not been sufficient time to carry them out, nor has the effect of implanting the limb exactly in the midline been studied.

The experiments with superposed buds were made in the same way as the above, except that the mesoderm of the host was not excised. In the case of half buds, more combinations are possible, as described in the section dealing with this group. Both here and in the superposition experiments all possible positions with regard to the placement of the graft within the limitations stated above were experimented with. Relations of harmony and disharmony proved to be the same here as in the case of whole buds.

The total number of cases of which records have been kept is 462. The analysis is based, however, upon the 271 individuals which yielded positive results. The identity of the individual cases has been maintained by rearing each in a container by itself and keeping a separate history of each. These histories consist in notes and in sketches made from time to time directly from the living specimens, mostly with the aid of the camera lucida.

In dealing with so large a mass of material it has of course been necessary to select typical cases for presentation, and in order not to interrupt the continuity of the general account, the individual histories, as far as given, have been gathered together in an appendix. The main body of the paper has been divided in

accordance with the outline presented above. The larger groups of experiments have been considered apart from each other, and each subgroup is treated in a special section. The peculiar features of each of the larger groups have been considered at the beginning, and the results of the experiments summarized separately at the end of each main section. The more general questions are treated in the final chapter.

It has been thought best to provide numerous illustrations in order to avoid lengthy descriptions. Since it was not possible to keep a complete pictorial history of each case, those were selected for drawing that promised typical or otherwise interesting results. Unfortunately, however, it was not always possible to predict what the outcome of an experiment would be, so that some important cases were not drawn in early stages, while others of less interest were.[7]

GENERAL FEATURES OF THE DEVELOPMENT OF THE TRANSPLANTED BUDS

The development of the transplanted limb buds must now be considered in comparison with normal development. When the normal limb bud appears it is a round prominence just below the pronephros. It soon becomes more sharply marked off from the background and begins to 'point' dorsoposteriorly.[8] The radial border of the fore arm and hand is at first ventrolateral, then ventral, and the first digits to arise are the first and second. The third and fourth digits appear later on the dorsal border of the hand, so that there is never any difficulty in distinguishing the ulnar from the radial border unless the third and fourth digits are entirely suppressed. The palmar surface of the hand faces at first ventromedially and later medially.

The transplanted limbs, both heterotopic and orthotopic, give evidence of their orientation early in development, inasmuch

[7] Almost all of the preliminary sketches and many of the finished drawings were made by Miss Lisbeth Krause. The former, which were pencil sketches, had to be redrawn for reproduction. For this part of the work and also for a number of the original drawings I am indebted to Mr. A. Hemberger and Mr. H. D. Rhynedance.

[8] Harrison, '18, p. 419.

as their direction of 'pointing' is determined principally by the bud itself. In two of the combinations (homopleural dorsoventral and heteropleural dorsodorsal), they point anteriorly or dorsoanteriorly; in the other two (homopleural dorsodorsal and heteropleural dorsoventral) posteriorly or dorsoposteriorly like the normal. The subsequent development in the latter case is normal, but in the former there is a tendency for the limb to stick out more sharply to the side or to rotate more or less from the position in which it would be found were the position determined entirely by the orientation of the bud itself. Nevertheless, the palm tends to face ventromedially, or else the limb is so rotated that it faces more ventrally or anteriorly. In order to determine whether the limb is right or left, it is necessary to be able to distinguish between the palm and the back of the hand, which is not always so simple as it might seem. It can usually be done, however, by noting the digits, which are frequently slightly flexed. When there is uncertainty, it is necessary to resort to sections, in which case there is no difficulty in distinguishing between the two faces, because of the much greater thickness of the soft parts on the flexor surface of the skeleton.

The duplicities that arise are of all grades and kinds, and occur in very different proportions in the several experiments. Sometimes they make their appearance very early, sometimes late in development. In the orthotopic grafts reduplication is far more common when the developing limb and the substratum are of opposite sides. In such cases the doubling member nearly always appears as a bud posterior to the main limb, growing there into a limb of proper asymmetry. The extent of reduplication may include the whole limb from the shoulder down, or only certain of the digits. The duplicate limb is as if it were mirrored from the original in a plane which is perpendicular to the plane of the proximodistal axes of the two limbs[9] and which cuts the axes of the two limbs at their junction, at an angle which varies from almost 0° to 90°. In the former case the two members are almost parallel, in the latter they diverge in the opposite direction at almost 180°, the mirror plane bisecting the angle between them

[9] Bateson, Materials for the Study of Variation, p. 479.

(fig. 3). In the present paper the relation of the mirror plane to the long axis of the limb has not been taken into account for purposes of description, the relation only to the dorsopalmar and the radioulnar axes being stated; i.e., the degree of divergence of the two members is not taken into account. Thus, when the mirror plane is parallel to the radioulnar axis, the limb is said to

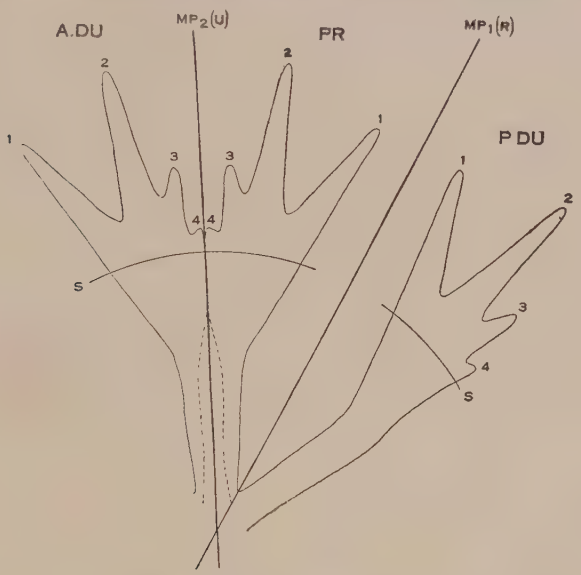


Fig. 3 Diagram showing mode of reduplication. *PR*, primary limb; *P.DU*, posterior reduplicating member; *A.DU*, anterior reduplicating member; $MP_1(R)$, primary (radial) mirror plane; $MP_2(U)$, secondary (ulnar) mirror plane; *1* to *4*, first to fourth digits, respectively. *S*, location of section shown in figure 4B. Dotted lines show the outlines of limbs as they would have been had there been no coalescence.

be mirrored in a palmar or a dorsal plane, according as the palms or the backs of the hand face one another; when the plane is parallel to the dorsopalmar axis, the mirroring is in a radial or an ulnar plane, according as the radial or ulnar borders of the limb face one another (fig. 4, *A*). Intermediate planes are described as radiodorsal, ulnopalmar, etc. (fig. 4, *B*). No attempt has been made for the present to measure accurately the angles of mirroring. It has been found, in agreement with Bateson, that

when there is a double reduplication, then the two mirror planes intersect at the bifurcation in a line perpendicular to the proximo-distal axes; i.e., so that with reference to the radioulnar and dorso-palmar axes the planes of reflection face one another (fig. 4). Considerable deviation from this rule has, however, been noted in certain cases, and the amphibians do not seem to follow it with the same regularity as the arthropods, according to Bateson.[10]

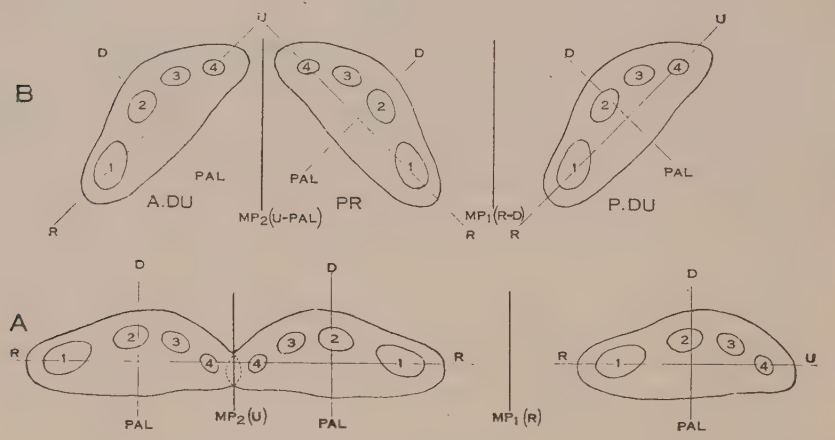


Fig. 4 Diagram of reduplication, sectional view. In A the mirror planes are radial (MP_1) and ulnar (MP_2), and a certain amount of coalescence between the primary and the anterior reduplicating members is shown, as in figure 3. In B the mirror planes are radiodorsal (MP_1) and ulno pulnar (MP_2). *D*, dorsal; *PAL*, palmar; *R*, radial; *U*, ulnar.

EXPERIMENTAL

A. Limb buds implanted in abnormal location—heterotopic transplantations

In nearly all of the experiments in this group the limb bud was implanted on the flank of the embryo at the ventral border of the myotomes between the region of the fore and hind limbs. In a few cases it was placed on the side of the head between the eye and the ear, but the grafts were absorbed in all of these except

[10] Op. cit., p. 552.

one, which yielded an imperfect appendage. They need not be considered separately here, though a more extensive series of experiments of the latter type would probably yield different and more interesting results.

The limb buds transplanted to the flank of the embryo are placed in an environment similar to that of the normal fore limb, as far as relations to the body wall and muscle plates are concerned, though they lack the specific blood supply and innervation of the limb region. Consequently, a very high percentage

TABLE 1

Heterotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Positive[1]	Number	Per cent	Number	Per cent	Number	Per cent
Hom. dd	19	7	3	42.8	0	00.0	4	57.1
Hom. dv	31	12	0	00.0	11	91.7	1	8.3
Het. dd	28	10	8	80.0	0	00.0	2	20.0
Het. dv	60	16	1(?)[2]	6.3(?)	7	43.8	8	50.0
Total	138	45	12	26.7	18	40.0	15	33.3
Average of percentages				32.3		33.9		33.9

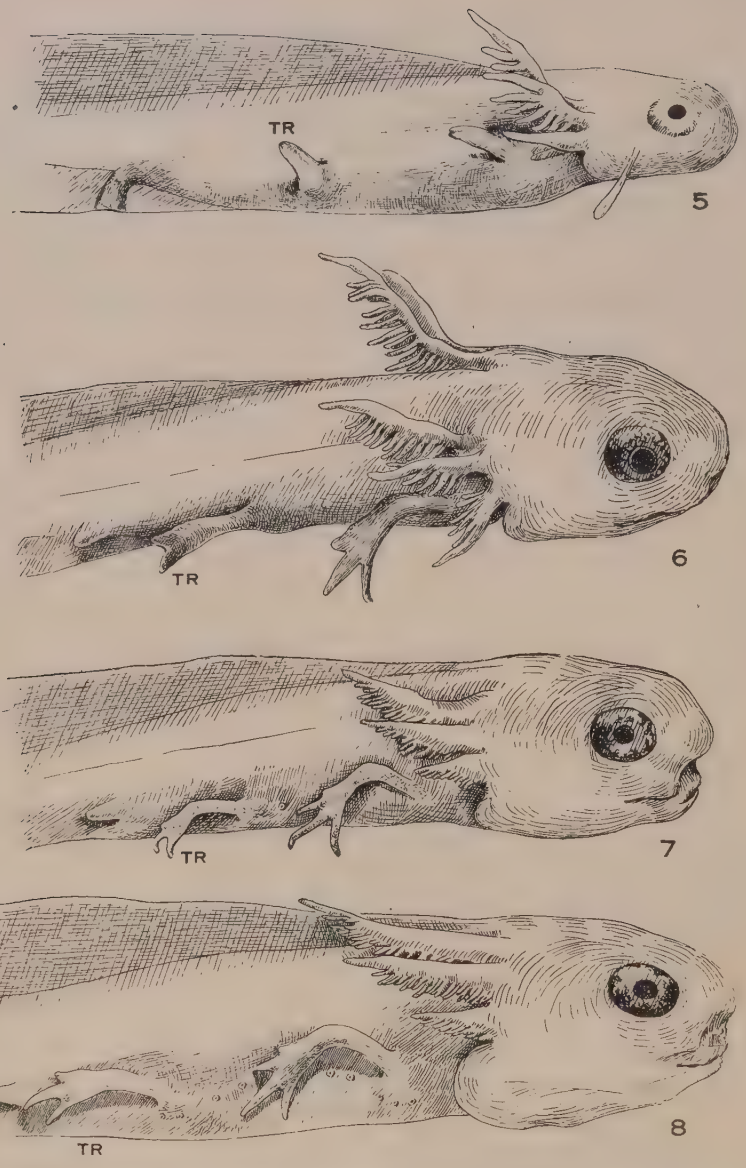
[1] Excluding all cases where death occurred prematurely or where the grafted limb was resorbed or remained rudimentary. Percentages in all tables have been calculated on the basis of positive experiments.

[2] There is evidence that in this case there was an error in the orientation of the bud and that it should therefore be classed in the group het. dd.

of cases yielded only abortive limbs, and those that did develop rarely showed any functional activity.[11] There is also greater difficulty in securing good healing of wounds in the intermediate region, so that a larger proportion of the cases died early. In many of these cases there is obviously some interference with the development of the pronephric duct, which becomes blocked. The secretion which accumulates causes the formation of a cyst of considerable size, which may interfere with the development of the limb bud.

The results of the experiments are summarized in table 1.

[11] Cf. Detwiler, '19 and '20.



Figs. 5 to 8 Heterotopic transplantation of fore limb; right limb to right side (*hom.dd.*). *TR*, transplanted limb. × 10.

Fig. 5 Exp. Tr. E. 148, eight days after operation.

Fig. 6 Same, twenty days after operation.

Fig. 7 Same, twenty-eight days after operation, drawn from preserved specimen.

Fig. 8 Experiment Tr. E. 154, drawn from preserved specimen, killed twenty-two days after operation.

1. Homopleural transplantations, normal or dorsodorsal orientation. Nineteen cases were operated upon in this way (table 1). In all of the cases where observations are recorded (thirteen in number), the limbs, in the course of their development, gave evidence of their original orientation, in that they pointed posteriorly or dorsoposteriorly when they first began to grow out (fig. 5). In the three cases that gave rise to single limbs they contin-

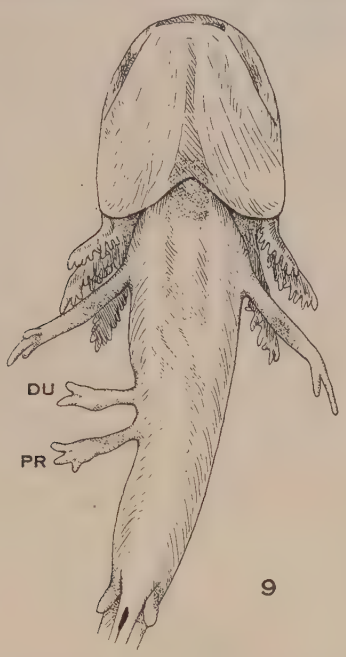
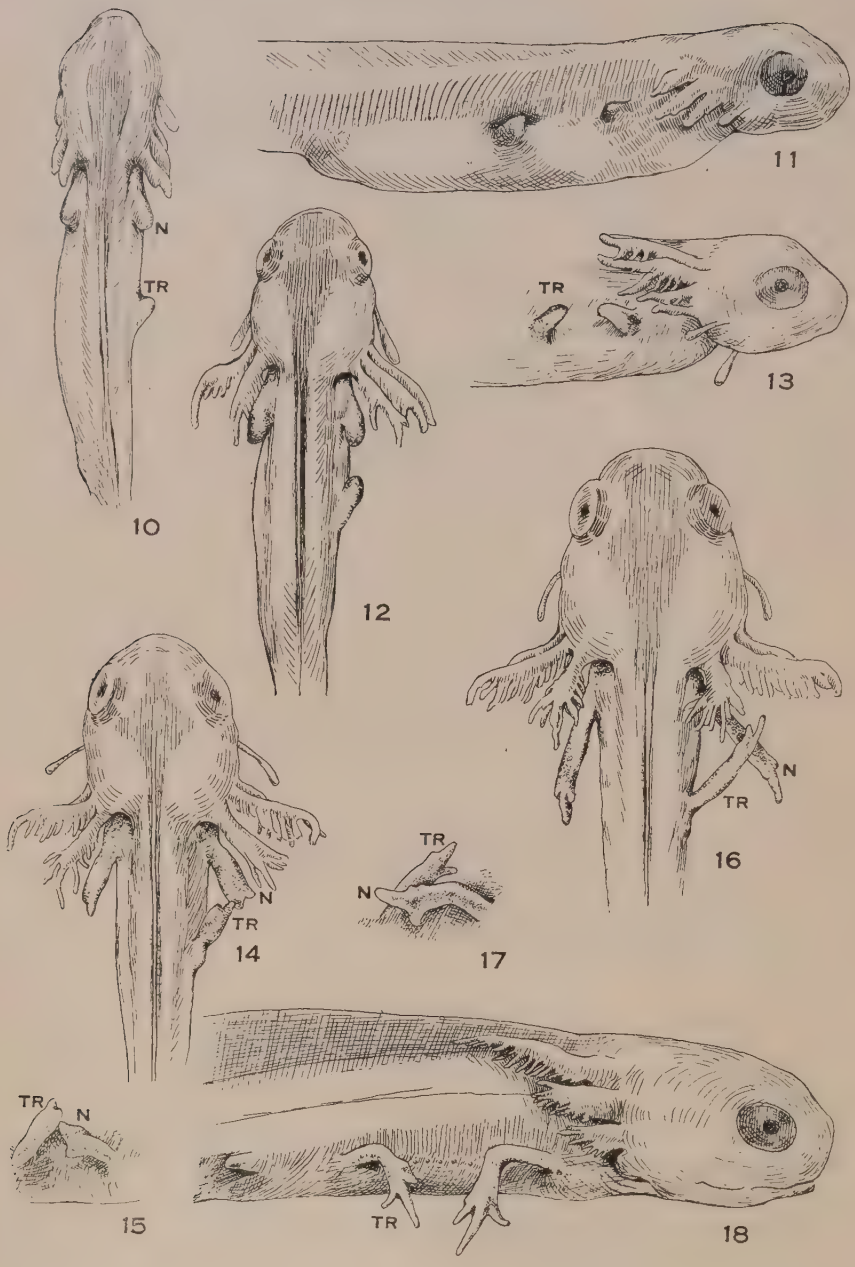


Fig. 9 Heterotopic transplantation (*hom.dd.*), showing twin limbs from one implanted bud; *PR*, primary member; *DU*, reduplicating member. Exp. Tr. E. 182. × 10.

ued their growth in this direction, developing almost exactly like the normal (figs. 6, 7, and 8). Likewise in the four cases that gave rise to double appendages, the transplanted buds first began to grow in a dorsoposterior direction, and only later did the reduplicating buds appear on the anterior border of the original limb. The original bud developed in each case into a limb of the same side, and the reduplicating buds became limbs of opposite asymmetry (fig. 9). Histories of typical cases are given in the appendix (p. 119).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15
TR
18

2. Homopleural transplantations, inverted or dorsoventral orientation. Thirty-one experiments of this kind were made, with results as shown in table 1. Of the twelve cases yielding positive results, one[12] gave rise to a pair of limbs and the others to single limbs in which the asymmetry was reversed; i.e., the right limb bud when placed upside down on the right side of the body gave rise directly to a left limb. Even in the case which showed reduplication the primary limb of the pair became reversed. In all of the cases where limbs resulted, the initial direction of pointing was anterior or dorsoanterior (figs. 10 and 11); i.e., nearly the opposite of normal. In four other cases this was also true. In only four cases is the direction of pointing recorded as posterior, and from these nothing definite was developed. All limbs which developed continued their growth in the same general direction, sometimes being directed more dorsally and sometimes more sharply anteriorly (figs. 12 to 17). They also showed the tendency to project more directly to the side than the normal limbs. The final posture assumed by these appendages varies considerably and does not seem to be dependent upon the degree of development attained by the appendage. Two cases, each having perfectly developed hands, exhibit the following extreme conditions: One[13] is practically a perfect mirror image of the normal right limb both as regards form and posture (fig. 18). The

Figs. 10 to 17 Heterotopic transplantation of fore limb; right limb bud to right side inverted (*hom.dv.*), Exp. Tr. E. 219. *N*, normal limb, right side; *TR*, transplanted limb. × 10.

Fig. 10 Dorsal view, five days after operation.

Fig. 11 Lateral view, same age.

Fig. 12 Dorsal view, eight days after operation.

Fig. 13 Lateral view, same age.

Fig. 14 Dorsal view, twelve days after operation.

Fig. 15 Lateral view of limbs only, same age.

Fig. 16 Dorsal view, sixteen days after operation.

Fig. 17 Lateral view, same age.

Fig. 18 Heterotopic transplantation; right limb to right side inverted (*hom. dv.*), Exp. Tr. E. 139; drawn from specimen preserved twenty-eight days after operation. × 10.

[12] Tr. E. 220.

[13] Tr. E. 139.

upper arm runs dorso-anteriorly and laterally. The elbow bend is somewhat less than 90° and the fore arm and hand extend antero-ventrally and laterally. The extensor surface of the elbow-joint faces dorsally and slightly anteriorly and medially. The palm of the hand faces medially, anteriorly, and slightly ventrally.

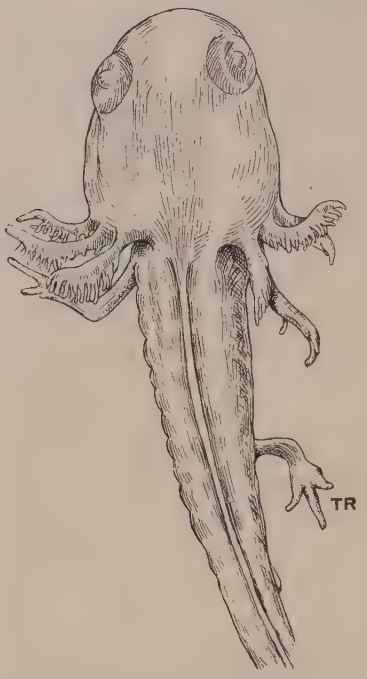


Fig. 19 Heterotopic transplantation (*hom.dv.*), Exp. Tr. E. 140; drawn from specimen preserved twenty-eight days after operation. *TR*, transplanted limb. × 10.

The other case[14] has its upper arm transverse and horizontal, and its fore arm extends ventroposteriorly at an angle of less than 45° to the horizontal axis (fig. 19). The palm looks ventrally and anteriorly. In order to bring this limb into the position of the former, it would have to be rotated about the axis of the humerus 45° or more and then adducted dorsoanteriorly at the shoulder-joint through about 30°. The difference in position assumed by the limbs in the various cases is thus due to differ-

[14] Tr. E. 140.

ences in the amount of rotation, etc., undergone during the later stages of development.

Histories of these cases are given in the appendix (p. 120).

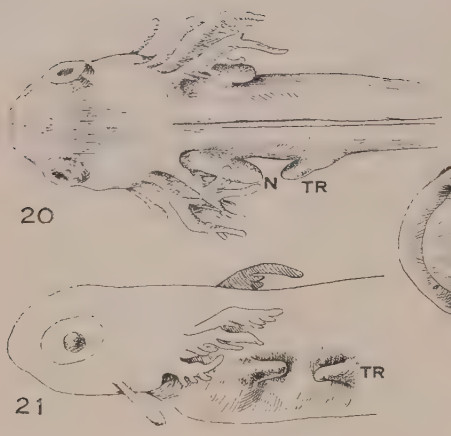
3. Heteropleural transplantations, dorsodorsal orientation. Twenty-eight experiments in this class have been made (table 1). Five of these died prematurely, and in twelve the tissue was either resorbed or failed to develop beyond the nodule stage. In one case[15] the bud developed into a stump about as long as the upper arm, but without digits. Two cases gave double limbs and eight developed into limbs which preserved their original prospective asymmetry. Two other cases may belong in this category, one in which the original orientation of the bud is recorded as uncertain[16] and another[17] in which it is recorded as dorsoventral probably by mistake.

In the development of the limb buds in this group twenty-one, in addition to the two doubtful cases just mentioned, are recorded at first as pointing in an anterodorsal direction, thus preserving their original tendency in this respect. In the eight cases in which the pointing was slight and in the five in which no definite pointing was observed the limbs were abortive or resorbed.

In the eight cases where single limbs of the side of origin developed they retained their posture, developing as nearly exact mirror images of the normal fore limb of the side to which they were transplanted (figs. 20 to 23). The elbow-joint points dorsoanteriorly, though varying somewhat, and the palm of the hand faces ventrally, medially, and anteriorly (figs. 24 and 25). Individual cases show variations similar to those observed in the previous group. It is a striking fact that the general type of development is the same here in the heteropleural non-inverted buds as in the homopleural inverted bud, which shows that both the posture and the asymmetry of the limb depend upon some reaction between the bud and its new environment. (For case histories see p. 121.)

The cases which showed reduplications, but two in number, differ considerably from one another. In the first[18] growth was slow and the resulting limb short with irregular reduplications

[15] Tr. E. 118. [16] Tr. E. 117. [17] Tr. E. 113. [18] Tr. E. 119.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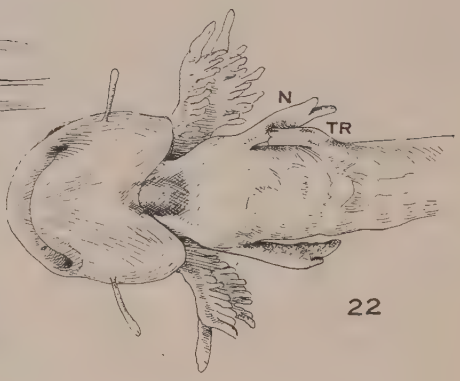
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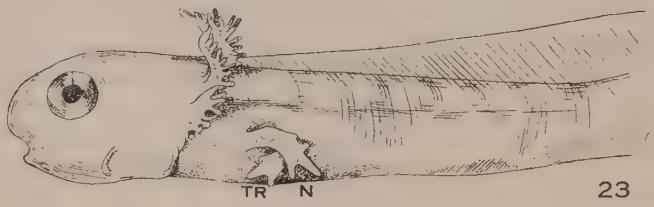
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N
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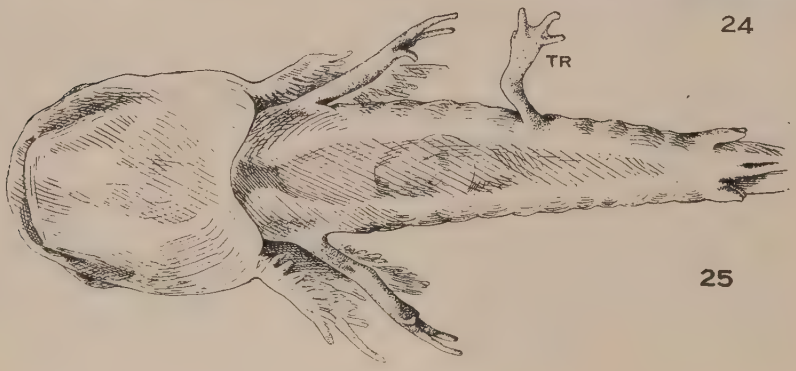
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in the hand, so that right- or left-sidedness could not be determined. In the other[19] the limb developed promptly and formed a duplicate member (fig. 26), which was first seen at ten days and

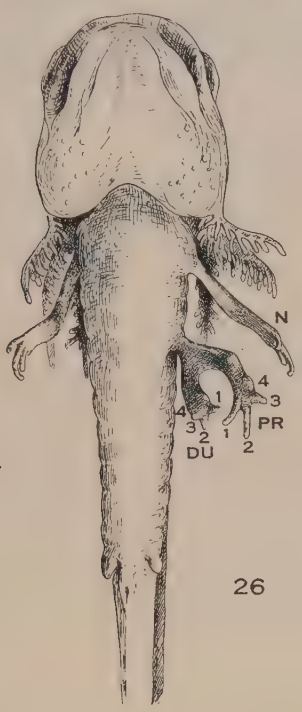


Fig. 26 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 127. Drawn from specimen preserved twenty days after operation. *PR*, primary; *DU*, reduplicating member; *1* to *4*, digits.

Figs. 20 to 23 Heterotopic transplantation of fore limb; right limb bud to left side (*het.dd.*), Exp. Tr. E. 227. *N*, normal left limb; *TR*, transplanted limb. × 10.

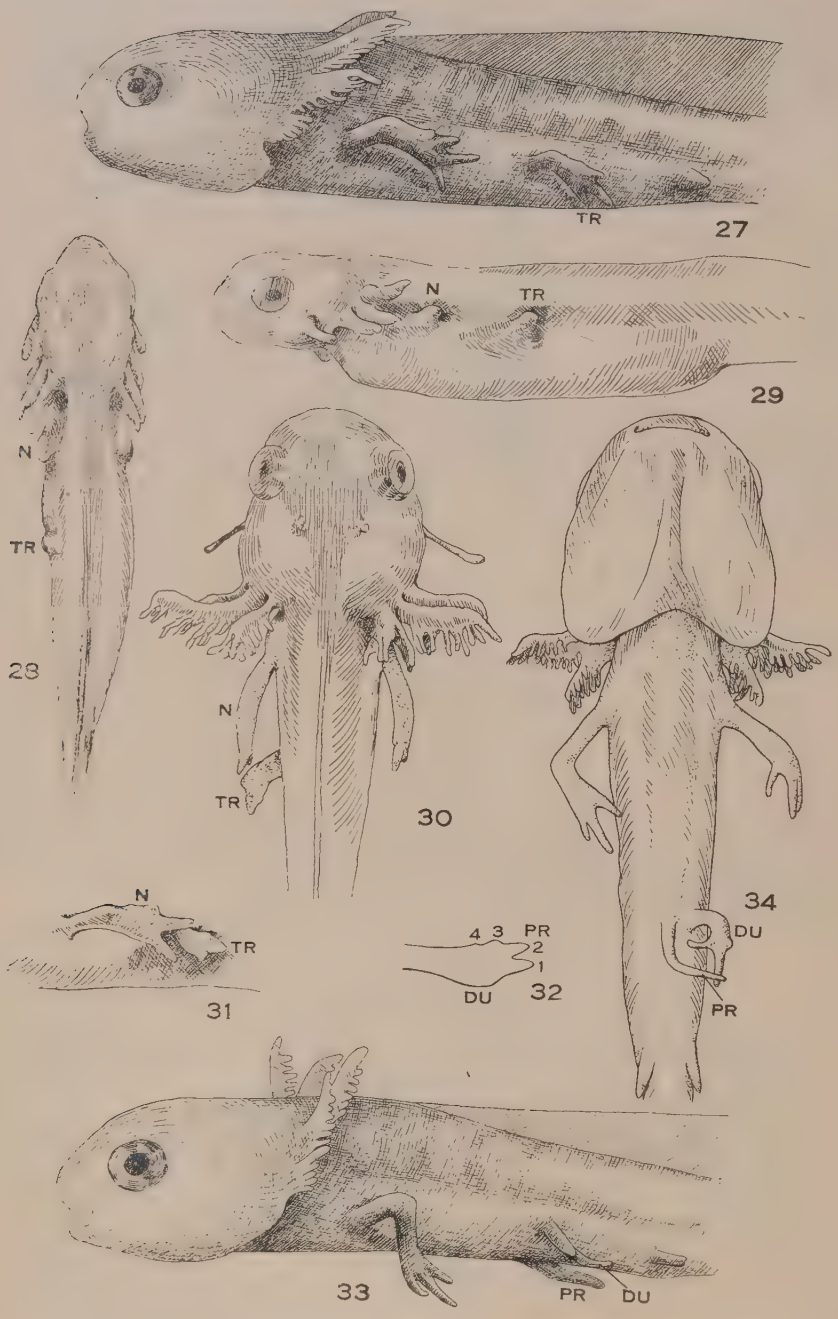
Figs. 20 and 21 Dorsal and lateral views, respectively, eight days after operation.

Fig. 22 Ventral view, thirteen days after operation.

Fig. 23 Lateral view, seventeen days after operation.

Figs. 24 and 25 Heterotopic transplantation (*het.dd.*), Exp. Tr. E. 107. Lateral and ventral views, respectively, of preserved specimen, twenty-six days after operation. × 10.

[19] Tr. E. 127.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32
PR
DU
33

which developed ultimately into a left limb, the mirror image of the primary member, having been reversed from the original prospective asymmetry of the transplanted bud.

4. Heteropleural transplantations, dorsoventral orientation. Sixty operations were done in this series. For some unknown reason a very large proportion (twenty cases) died prematurely and sixteen of the survivors yielded only abortive limb buds, leaving only twenty-four available for consideration. Eight of these are recorded as imperfect, six produced reduplications to some degree, and nine, single limbs of reversed asymmetry. Several of the latter were somewhat defective and others showed slight reduplications. Several cases which are exceptional will be considered below.

In the cases where single limbs arose, development took place in a manner fundamentally like that of the limb buds normally oriented (*hom. dd.*). As the buds grew out, they began to point in a posterior direction, and so continuing, developed into limbs in normal posture (fig. 27). There was, however, less regularity than in the homopleural dorsodorsal group. The direction of pointing was not always dorsoposterior, as in the normal limb, but was in many cases inclined more ventrally. There are records of pointing in all of the positive experiments and in many of the negative. In only three cases is the direction recorded

Fig. 27 Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 193. Preserved specimen killed twenty-four days after operation. × 10.

Figs. 28 to 32 Heterotopic transplantation of fore limb; right limb bud to left side inverted (*het.dv.*), Exp. Tr. E. 217. *N*, normal left limb; *TR*, transplanted limb; *PR*, primary member; *DU*, reduplicating member; *1* to *4*, numbers of digits.

Fig. 28 Dorsal view, five days after operation.

Fig. 29 Lateral view, five days after operation.

Fig. 30 Dorsal view, fifteen days after operation.

Fig. 31 Lateral view of limbs, fifteen days after operation.

Fig. 32 Limb showing beginning of reduplicating digits (*DU*) on ventroanterior border (from a free-hand sketch nineteen days after operation).

Figs. 33 and 34 Heterotopic transplantation (*het.dv.*); right limb bud to left side. Exp. Tr. E. 163. Anomalous result. Primary member (*PR*) defective; reduplicating member (*DU*) reversed. Lateral and ventral aspects, respectively, drawn from specimen preserved thirty-nine days after operation. × 10.

as dorsoanterior; one of these died early and the other two gave rise to imperfect limbs with indeterminate asymmetry.[20]

The individual cases in which limbs of opposite asymmetry developed were rather more irregular than in the preceding groups, though the best cases gave perfect reversed appendages. In addition to the ones included in the tabulation, there is one other case that probably belongs in this category. It is one in which the orientation of the bud at the time of transplantation is recorded as uncertain.[21] The limb that developed is a perfect one of reversed asymmetry in nearly the same posture as the normal limb of the side to which it was transplanted. It showed an unusual amount of motility. In one case, included in the records of this group,[22] the transplanted bud developed into a normal limb of the side from which it was taken. It is believed, however, that a mistake was made in recording the operation in this case, and that probably in reality the orientation of the limb was not inverted. The direction of pointing, as observed on the third and fifth days after the operation when the limb bud is recorded as pointing anteriorly, is evidence, though not absolutely conclusive, that an error has been made. If this interpretation is correct, the case would not be exceptional, but would accord with the eight cases described in the previous section.

In the eight cases in which reduplications occurrred, the early stages of development were like the normal (figs. 28 and 29), the reduplicating buds not being noted until at least twelve days after the operation. Three individuals showed distinctly that the primary limb was of reversed asymmetry. In one case it was so imperfect that it could not be determined to which side it belonged, but the reduplicating limb was sufficiently developed to show that it was of the same side as the bud was originally, indicating that the original member was in all probability reversed. Another case[23] gave a limb with nearly symmetrical reduplication in the hand without anything to indicate which member was primary (figs. 31 and 32). Two long radial digits are present in the middle and two short ulnar digits on each side. Still another case[24] gave a very peculiar result. The primary

[20] Tr. E. 108 and 203. [21] Tr. E. 109. [22] Tr. E. 113. [23] Tr. E. 217.
[24] Tr. E. 163.

limb bud developed into a long almost filiform structure, without digits, that grew posteriorly on the ventral side of the body not far from the midline. Twenty days after the operation a second bud was noticed dorsal to the original, and this developed into a somewhat peculiarly placed limb. The upper arm runs transversely and the palm of the hand faces dorsomedially (figs. 33 and 34). This limb is clearly a left; i.e., its original prospective asymmetry has been reversed. It therefore constitutes an exception to the rules, not only because of the position of the hand, but also because of its particular asymmetry; for the original (filiform) member should have been reversed (a left), and the second one reversed back again to the original asymmetry. However, the fact that the latter developed at such a considerable distance from the original member, might be regarded as indicating that it was beyond its sphere of influence, perhaps having been split apart from it at an early stage, and that it remained therefore as of the same side. Several cases of regeneration after extirpation of half buds and of transplantation of half buds gave analogous results (fig. 132).[25]

5. The shoulder-girdle in heterotopic transplantations. The limb-girdle in the hetrotopic transplantations is developed in more or less reduced condition, as was first shown by Braus ('09) in the anurans. Detwiler ('18) has studied this question in Amblystoma, and has found that the degree of development of the girdle is dependent upon the size of the graft and the region from which it is taken, the scapula and suprascapula being localized in the tissue dorsal to the normal limb bud and the coracoid in that ventral to it.[26] The form of the reduced girdle derived

[25] Cf. Harrison, '18, p. 441 (Exp. Rem. E. 17 and H. R. E. 10), and page 135 of the present paper (Exp. H. R. E. 20).

[26] It is a curious fact that in the embryo the limb-girdle has undoubtedly the character of a mosaic, without totipotence of its parts, while in the adult Triton, according to Tornier ('06), Fritsch ('11), and Kurz ('12), a small portion of the shoulder-girdle can regenerate the whole, including the fore limb. According to the two last-named investigators, even if the whole girdle is removed, it will be regenerated together with the free appendage. Kurz has found that this holds for both shoulder and pelvic girdles but that removal of the sacral portion of the vertebral column prevents regeneration. In the anurans, according to Braus ('06), there is considerable variation in the regenerative powers of the limbs in early stages.

from the usual round disc (limb bud) is roughly triangular, as shown in the figure of Detwiler's model (his figure 28), with a ventral process projecting anteriorly, to be identified as a rudimentary coracoid, and a dorsal process, which includes the rudiment of the scapula. In the normally oriented grafts (homopleural dorsodorsal) these processes point anteriorly, with a single process projecting posteriorly slightly behind the glenoid cavity. This shows clearly in two cases.[27] The question now arises whether the girdle follows the rules governing the asymmetry of the free limbs. The results, in the main indicate that such is the case, though the girdle developed is often so small and rudimentary, that it is not possible to determine to which side it belongs. In the inverted homopleural grafts, which give rise to reversed limbs, the girdle also seems to be reversed. This is true in four cases out of the five examined in serial sections.[28]

Among the heteropleural dorsodorsal transplantations, five cases have been examined in sections. In two of them[29] with well-developed glenoid cavity, the girdle cartilage is mostly ventral and posterior to the joint. This probably represents a coracoid with asymmetry corresponding to that of the free limb. One case,[30] with the cartilage projecting both anteriorly and posteriorly from the cavity, gives no evidence as to the side to which it belongs. One is too rudimentary,[31] and one seems to have had its asymmetry reversed,[32] though the limb is not reversed. In the two dorsoventral heteropleural transplants which have been studied in sections, the side to which the girdle belongs cannot be determined. Other cases from among the earlier experiments, where in most instances the size of the transplanted bud was small, are inconclusive. On the whole, the cases where the asymmetry can be determined with any degree of certainty seem to follow the rules. Only a single case thus far examined is clearly exceptional.

[27] Tr. E. 148 and 154.
[28] Tr. E. 135, 136, 139, and 140.
[29] Tr. E. 124 and 169.
[30] Tr. E. 120.
[31] Tr. E. 127.
[32] Tr. E. 107.

6. Summary of the results of heterotopic transplantations. A survey of all the experiments in this group brings out the following facts:

Implanted in dorsodorsal orientation, a limb bud gives rise to an appendage of its original prospective asymmetry, whether placed on the same or opposite side of the body. Such appendages have a normal posture when placed on the same side of the body from which they were taken, but when placed on the opposite side they mirror approximately the limb of that side, though they often become rotated to quite different postures. Implanted in inverted (dorsoventral) position, a limb bud gives rise to an appendage of reversed asymmetry whether placed on the same or opposite side of the body. When placed on the same side, such appendages mirror the normal limb of that side, but when grafted on the opposite side, they assume a posture approximately identical with that of the limb of that side.

Limbs implanted in any of the four positions here studied may produce reduplications. As far as it has been possible to determine, the primary limb of the pair is then of the same side as a single limb would be according to the foregoing rules. The reduplicating limb has been found to be, with a single exception, the mirror image of the first.

Limbs that are grafted in abnormal location have at best very incomplete function and are often apparently entirely immobile. They usually do not become so large as those that are implanted in normal location, and they show defects and evidences of atrophy much more frequently.

B. Limb buds implanted in natural location—orthotopic transplantation

In these experiments the limb bud of the host was first removed and then either put back in place, or else a bud from another embryo was grafted into the wound. In all of the earlier cases the wound bed was not cleaned after removal of the bud, so that some cells from the host were left to mingle with the tissues of the transplanted limb rudiment. The later experiments, with

but few exceptions, were carried out under precautions necessary and sufficient to preclude contamination of this kind: the extirpated area was three and a half somites in diameter, and the bed of the wound was carefully scraped after removal of the bud.[33] The results were somewhat different (proportionately) in the

TABLE 2

Orthotopic transplantations. Summary of experiments

OPERATION	NUMBER OF EXPERIMENTS		SINGLE LIMBS NOT REVERSED		SINGLE LIMBS REVERSED		REDUPLICATED	
	Total	Positive	Number	Per cent	Number	Per cent	Number	Per cent
A. Wound bed cleaned and wound not less than $3\frac{1}{2}$ somites								
Hom. dd	9	9	9	100.0	0	00.0	0	00.0
Hom. dv	61	38	*10*[2]	*26.3*	1	2.6	27[1]	71.1
Het. dd	49	31	1	3.2	*5*[3]	*16.1*	25	80.6
Het. dv	26	16	0	00.0	15	93.8	1	6.3
Total	145	94	20	21.3	21	22.3	53	56.4
Average of percentages				31.6		28.8		39.6
B. Wound bed not cleaned								
Hom. dd	0	0	0		0		0	
Hom. dv	37	20	19[4]	95.0	0	00.0	1	5.0
Het. dd	17	13	2	15.4	3	23.1	8	61.5
Het. dv	21	15	0	00.0	8	53.3	7	46.7
Total	75	48	21	43.8	11	22.9	16	33.3

[1] Including three cases in which the primary bud righted itself by rotation and the duplicate is disharmonic.

[2] Limbs which became normal by rotation, including one case (I. E. 101) of hyperdactyly.

[3] Normal by resorption of original member of pair.

[4] One case included in which the posture of the limb was abnormal.

two classes of experiments and have been summarized separately in table 2 (A and B). The differences will be taken up in connection with the consideration of each of the subgroups.

7. Homopleural transplantations, dorsodorsal orientation. This is in reality merely a control experiment and is a test of the effect

[33] Harrison, '15 and '18, p. 422.

of the operation as such on the development of the limb. A fore limb bud is carefully excised and either replaced in the same wound or else engrafted in normal position in another embryo from which the limb bud had been previously removed.

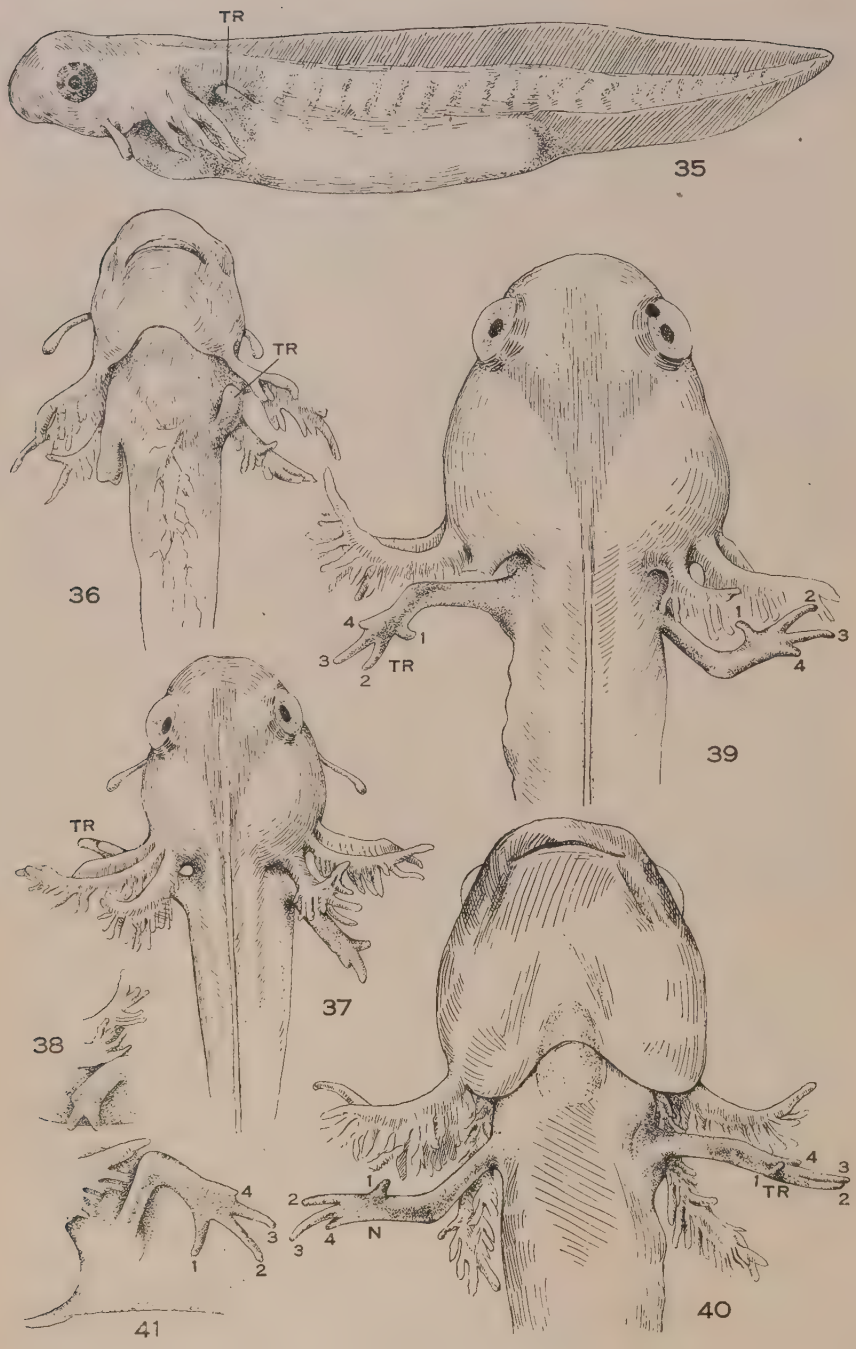
Only nine individuals were operated upon, in all of which the wounds were carefully cleaned. Normal limbs developed in all cases, though they were slightly retarded in the earlier stages of development in comparison with the unoperated limb of the opposite side. In six of the cases the pronephros was removed and in the other three it was left in. No difference was noted between the two sets. It may be safely concluded that the effect of the operation itself upon normal development is practically negligible.

8. Homopleural transplantations, dorsoventral orientation. In some of the cases of this series, as in the last, the limb bud was simply lifted and replaced after rotation through 180°. In the others the wound bed was first prepared in one embryo and the bud taken from another. The latter method is preferable and it was employed in all the later experiments.

The total number of experiments is one hundred and four, of which sixty-one were with cleaned wounds of proper size. The latter will be considered first, since the conditions of experimentation are more definitely known and there can be no doubt that the limbs were derived exclusively from the transplanted tissue.

Leaving out of consideration the twenty-three cases which died prematurely or gave rise merely to abortive or rudimentary limbs, there are thirty-eight cases which yielded positive results, as recorded in table 2A, The single limbs are in the minority and are of two kinds, reversed and non-reversed. The most remarkable case[34] (history on p. 124), which really gives the clue to the interpretation of the experiments of this group, is the one in which a limb of reversed asymmetry developed, a right limb on the left side, perfectly normal in form, function, and posture, as far as the last is possible on the wrong side of the body (figs. 35 to 41). The shoulder-girdle of this limb is also reversed and

[34] I. E. 64.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is quite separate from the rudimentary girdle developed from the tissues of the host (p. 59).

The ten cases in which normal non-reversed limbs developed are clearly contrary to the rule (p. 4). The records of these cases show that the end result is reached by a process of rotation at the shoulder-joint during development (figs. 56 to 58). They will be considered below (p. 40). The reduplicated limbs, of which there were twenty-seven, fall, like the single, into two groups. In the first the original bud developed into a limb of reversed asymmetry, while in the second it is not reversed.

Observations upon the earlier stages of the operated limbs show that in those cases which give reduplications, as well as in the case of the simple limb with reversed asymmetry (figs. 35, 42, 43, 49, 51, and 52), the original direction of pointing is either dorsal, anterior, or dorsoanterior, and more sharply lateral than normal. Likewise in the case of those that develop into single non-reversed limbs, the first pointing is more sharply lateral than normal, and also more dorsal, though only two[35] are recorded as pointing slightly anteriorly from the dorsal direction. This shows that the original tendencies of growth, immanent in the bud at the time of transplantation, are by no means inactive when it is in its new position. One or the other of two consequences of this growth tendency now ensues, indicating a sort of antagonistic reaction between the organization of the transplanted rudiment and that of the surrounding parts. The limb either continues to grow in an anterior or anterodorsal direction, in

Figs. 35 to 41 Orthotopic transplantation; left limb to left side inverted (*hom,dv.*), resulting in a normal right limb on the operated side. Exp. I. E. 64. *TR*, transplanted limb. × 10.

Fig. 35 Lateral view, five days after operation.

Fig. 36 Ventral view, ten days after operation.

Fig. 37 Dorsal view, sixteen days after operation; transplanted limb covered by gills.

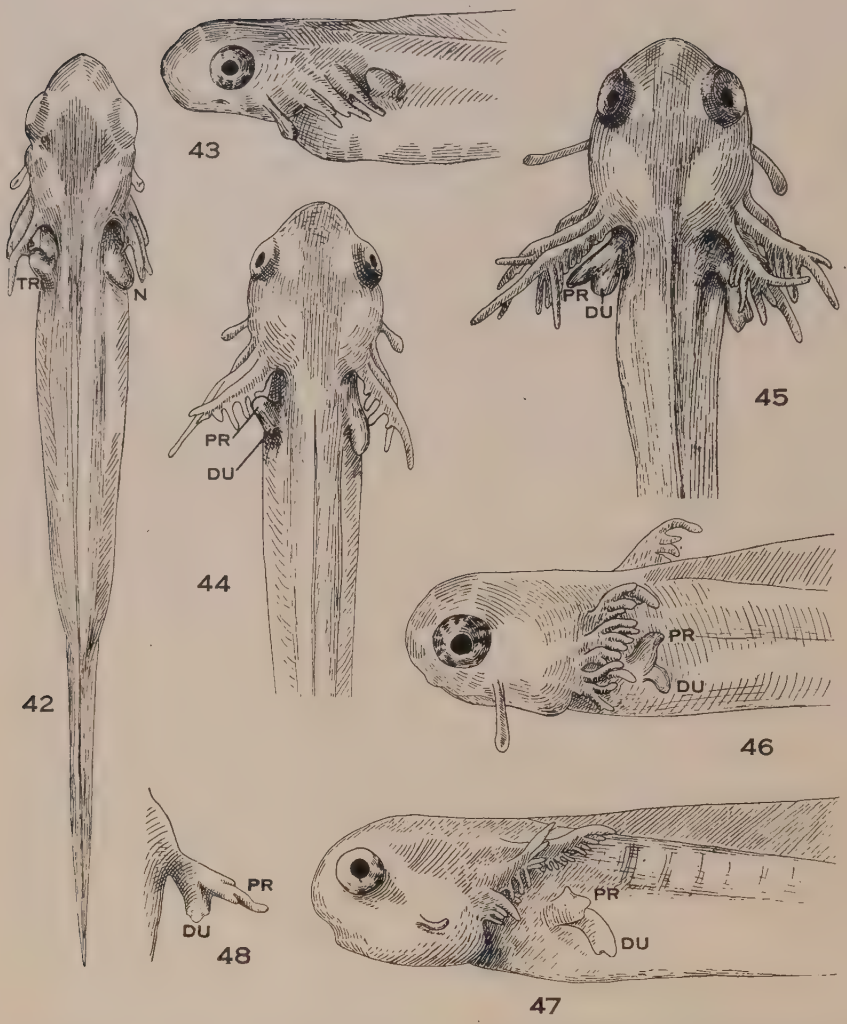
Fig. 38 Lateral view of limb, sixteen days after operation.

Fig. 39 Dorsal view, twenty-three days after operation.

Fig. 40 Ventral view, twenty-three days after operation.

Fig. 41 Lateral view, twenty-three days after operation.

[35] I. E. 49 and 94.



Figs. 42 to 48 Orthotopic transplantation; left limb bud to left side inverted (*hom.dv.*), resulting in duplicate limbs. Exp. I. E. 60. *N*, normal right limb bud; *TR*, transplanted bud; *PR*, primary member; *DU*, reduplicating member. × 10.

Fig. 42 Dorsal view, five days after operation.
Fig. 43 Lateral view, five days after operation.
Fig. 44 Dorsal view, seven days after operation.
Fig. 45 Dorsal view, twelve days after operation.
Fig. 46 Lateral view, twelve days after operation.
Fig. 47 Lateral view of specimen preserved eighteen days after operation.
Fig. 48 Ventral view of limb, preserved specimen.

which case its asymmetry is reversed (figs. 36 to 39), just as in the corresponding class of heterotopic transplantations, or it gradually rotates towards its normal position while retaining its original prospective asymmetry (figs. 56 to 59). In the former alternative duplicate limbs are nearly always formed. Only in the one case, referred to above, did a perfect single limb arise. In the other alternative single limbs usually arise, though some of the cases of reduplication certainly belong to this group.

In the duplicities belonging to the first group the original limb bud continues to grow in an anterior direction and ultimately becomes a reversed limb. After a time a reduplicating bud appears on the posterior border of the original bud (fig. 44) and in the clearer cases grows into a homopleural limb in approximately normal posture (figs. 45 to 48). The original bud becomes a reversed limb which, together with the reduplicating member, may form an almost symmetrical complex.

Twenty-four of the thirty-one[36] cases of reduplicated limbs are probably of this type. Fifteen are certainly so,[37] and in three others[38] that are very similar all that is lacking to place them unequivocally in this group is a definite observation as to which bud was the primary one; six more cases[39] may also be interpreted in the same manner, though they are not sufficiently clear to insure that this is the only possible interpretation.

The degree of reduplication varies here, as in the other groups of experiments, from the condition where almost the whole arm is involved to that in which the hand is only partly double. In three cases[40] the anterior bud was much reduced (p. 49), the posterior bud becoming a somewhat irregular homopleural limb. In eleven cases there is only one reduplicating appendage, which is always posterior to the primary (figs. 43 to 48), while in the remaining twelve[41] there are evidences of further doubling, usu-

[36] Four cases are considered here which are not included in the tabulation on account of the fact that the wound was only 3 somites in diameter (I. E. 39, 41, 44 and 45).

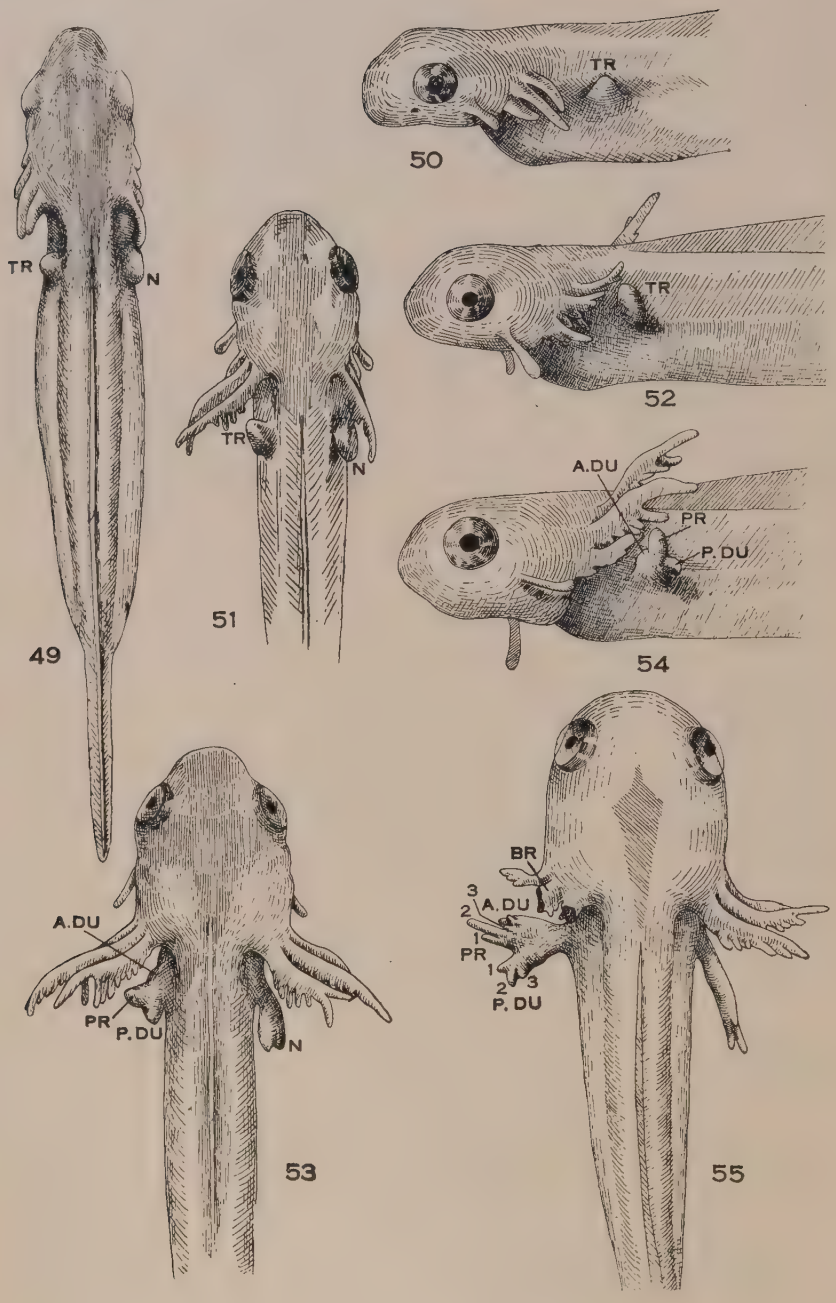
[37] I. E. 48, 60, 62, 63, 66, 72, 74, 75, 81, 85, 87, 89, 91, 92, and 96.

[38] I. E. 44, 45, and 52.

[39] I. E. 39, 68, 70, 93, 100, 102.

[40] I. E. 92, 93, 100.

[41] I. E. 39, 45, 62, 63, 66, 72, 75, 81, 85, 87, 91, and 93.


TR
N
49
TR
N
51
TR
50
TR
52
A.DU
PR
P.DU
54
A.DU
PR
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N
53
BR
3
2
A.DU
1
PR
1
2
3
P.DU
55

ally on the anterior side of the original (figs. 49 to 55 and 61). When the latter condition arises and the anterior reduplicating member is sufficiently developed, it is seen that it, too, is mirrored from the original member and is homopleural (fig. 55). In one case there are three complete hands, one of which has two of its digits doubled.[42] The plane from which the posterior reduplicating member is mirrored in the final form of the limb varies from radial to dorsal (figs. 3 and 4) and is usually intermediate between these two extremes (p. 13). Nineteen cases follow this rule, three are indeterminate and there is only one positive exceptional case, in which the mirror plane is ulnodorsal.[43] When there is also an anterior reduplicating member, it is generally mirrored from a plane 180° around the limb axis from the first; i.e., ulnar, ulnopalmar, or palmar.

The reduplications belonging to the second group are more restricted and less certain of diagnosis. The limb bud retains its original prospective asymmetry, reaching an approximately normal position by rotation, and reduplication is much less extensive, involving in most cases the digits only (fig. 62). Three cases[44] almost certainly belong to this group, and there may be two others.[45]

Of the two remaining cases of reduplication, one died too young; in the second[46] the supernumerary limb was of the same side as the primary and was quite distinct from it. This is a very unusual condition, but the transplanted bud in this case was

Figs. 49 to 55 Orthotopic transplantation; left limb bud to left side inverted (*hom.dv.*), resulting in limb with two reduplicating members. Exp. I. E. 63. *N*, normal right limb bud; *TR*, transplanted left bud; *PR*, primary limb; *A.DU*, anterior, and *P. DU*, posterior reduplicating members. × 10.

Fig. 49 Dorsal view, four days after operation.

Fig. 50 Lateral view, four days after operation.

Fig. 51 Dorsal view, seven days after operation.

Fig. 52 Lateral view, seven days after operation.

Fig. 53 Dorsal view, ten days after operation.

Fig. 54 Lateral view, ten days after operation.

Fig. 55 Dorsal view of specimen preserved seventeen days after operation. Gills (*BR*) removed to show limb. *1* to *3*, numbers of digits.

[42] I. E. 87. [43] I. E. 72. [44] I. E. 86, 88, 90. [45] I. E. 41, 59. [46] I. E. 38.

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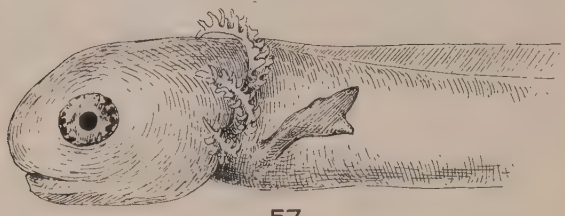

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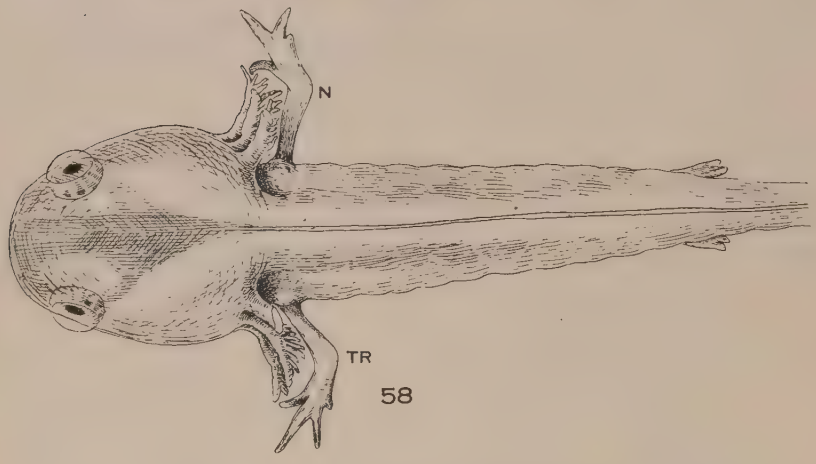

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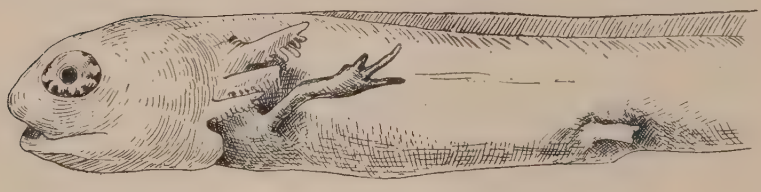

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larger than usual (four somites in diameter), and it is possible that the reduplicating bud, growing from near its anterior border, was uninfluenced by the primary limb and hence was not mirrored.

There remain for consideration those cases in which a single non-reversed limb developed. As in the other cases, the limb bud in these showed at first the consequences of abnormal orientation. When first observed it pointed more sharply laterally and more dorsally (less posteriorly) than normal. Two even pointed dorsally and slightly anteriorly. In the course of development the limb gradually changed its posture and ultimately came to a perfectly normal posture by a process of rotation at the shoulder-joint (figs. 56 to 59). Ten such cases were obtained,[47] though in several of them[48] it is possible that reversal may have been brought about by early reduplication and suppression of the original bud, as described in the next section (p. 49). In one of these[49] a supernumerary radial digit was present, but this is to be regarded as a case of hyperdactyly rather than one of mirrored reduplication. In one case[50] the limb which originally developed showed irregularities in the digits. The arm was then amputated above the elbow, and the appendage which regenerated was in every respect normal. This case is of considerable interest in showing that the abnormal condition which produces reduplication is not necessarily stamped upon the whole structure, but may be due to some local mechanical disturbance.

In reviewing this group of experiments, it is clear that the first two results, i.e., single reversed limbs and most of the reduplications, come under the same scheme. There is a primary reversal of asymmetry, without reduplication in the first case and accom-

Figs. 56 to 59 Orthotopic transplantation; left limb bud inverted (*hom.dv.*). Limb reaches normal posture by rotation. Exp. I. E. 49. *N*, normal (right) limb; *TR*, transplanted (left) limb.

Fig. 56 Eleven days after operation.

Fig. 57 Twenty-one days after operation.

Fig. 58 Thirty-eight days after operation.

Fig. 59 Preserved specimen, killed at thirty-nine days.

[47] I. E. 49, 55, 69, 71, 73, 77, 84, 94, 99, and 101.

[48] For instance, I. E. 77.

[49] I. E. 101.

[50] I. E. 71.

panied by reduplication in the second. In the latter the secondary bud, being the mirror image of the other, is again reversed back to the original prospective asymmetry of the transplanted bud. This then occupies an approximately normal position and may function to a considerable extent as a normal limb, though impeded by the connection with its mate. These two results are directly comparable to those of the heterotopic transplantations of the corresponding class (fig. 2).

The third result, in which normal homopleural limbs develop, reaching their normal position gradually by rotation, is fundamentally different. Here no reversal occurs; the limb bud begins its development as a self-differentiating system, but later, under the stress of the changed relation to its environment, it comes again into normal posture.

What determines whether the limb bud shall reverse its asymmetry or rotate back to its normal posture? The earlier experiments of this series afforded no satisfactory answer to this question. It was certainly not due to the size of the wound, the mode of preparation of the wound, the presence or absence of the pronephros, or the age of the embryo. What seemed most likely was that there were minor accidental differences in the amount of rotation to which the limb bud was subjected at the time of operation. It was conceivable, for instance, that if the disc were rotated anteriorly around the dorsal semicircumference of the wound a little less than 180° (fig. 60), the reversing effect of its organic environment might be lessened and the rotation back to normal position facilitated; in this case a normal non-reversed limb would result. If, on the other hand, the grafted bud were rotated 180° or slightly more, the reversing effect might be at a maximum and rotation most impeded, in which case a heteropleural limb or twin limbs would arise.

Experiments made in the spring of 1917 had for their main purpose the testing of this hypothesis. Operations were done in pairs; in one case the limb disc was rotated about the dorsal circumference in a posteroanterior direction slightly more than 180° and in the other slightly less, extremes being probably not more than 190° and 170°, respectively (see histories on pp. 125–6).

The results are not altogether conclusive, though they point to the correctness of the hypothesis. Twenty-three operations were done, of which seventeen yielded positive results. Ten cases harmonize with the hypothesis; four are doubtful, and three are contrary to it (figs. 61 and 62). When the difficulty of exactly estimating the degree of rotation is considered, many apparent exceptions must be expected, and a far greater number of experiments would be necessary to eliminate statistically the effect of this uncertainty. As a matter of fact, the records of the cases classed as surely exceptional give evidence that the amount

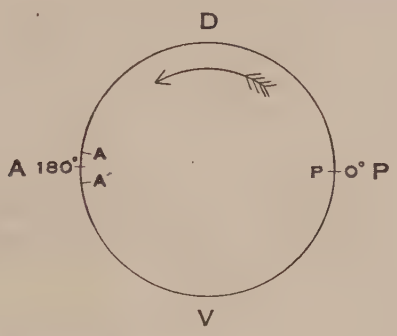


Fig. 60 Diagram showing difference in amount of rotation in two sets of experiments. The circle represents the left limb bud and the arrow the direction of rotation. *A, D, P, V,* the direction of the cardinal points of the embryonic body, anterior, dorsal, posterior, and ventral, respectively.

of rotation at the time of operation was probably not correctly estimated, for the first direction of pointing (p. 11) in all of these cases is not according to expectation.

The thirty-seven cases in which the wound bed was not entirely cleaned of mesoderm may now be considered. These show a marked contrast to those with cleaned wounds, inasmuch as there are very few reduplications and a very large proportion of normal non-reversed limbs. Thus out of twenty cases which yielded positive results eighteen or 90 per cent are normal, as compared with 23.8 per cent (ten cases) in the clean-wound class. Only one case (5 per cent) had a reduplicated limb, as compared with thirty-one duplicities (73.8 per cent) in the clean-wound class.

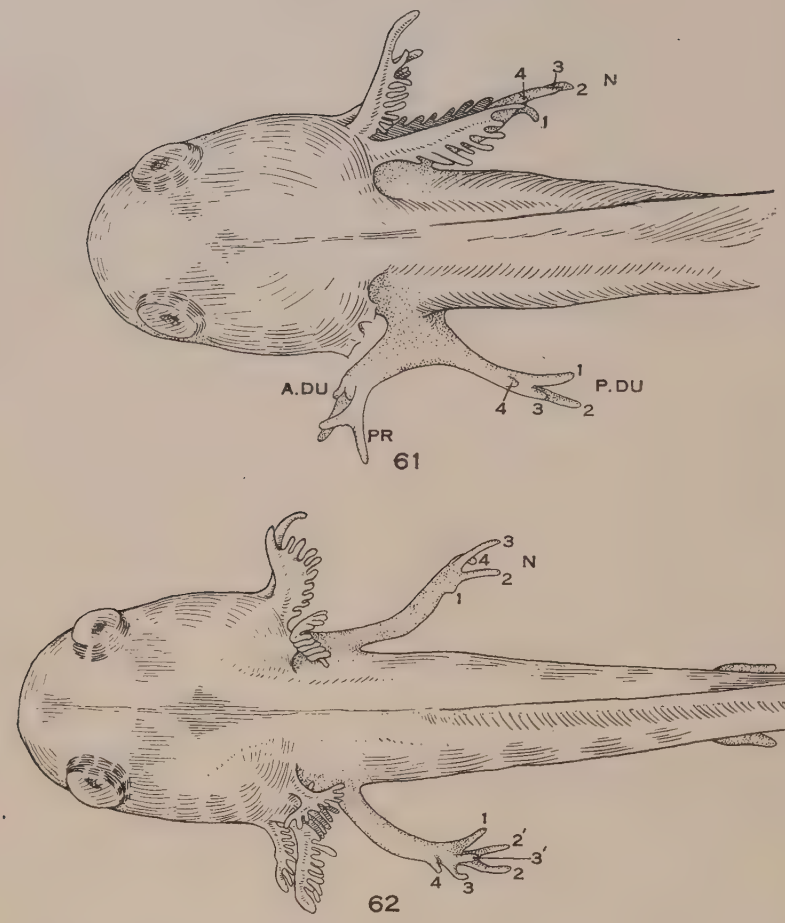
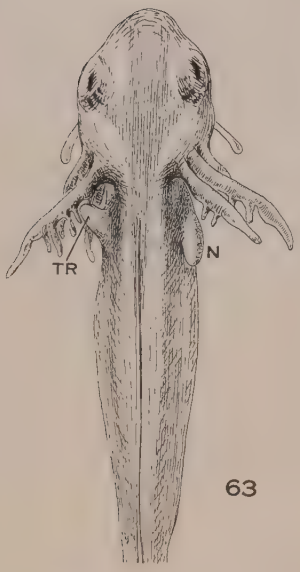


Fig. 61 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly more than 180° from its normal position, *i.e.* P to A′ in fig. 60. Exp. I. E. 85. Primary member (*PR*) is a right; posterior reduplicating member (*P.DU*) is a nearly normal left; anterior reduplicating member (*A.DU*) partly coalesced with primary.

Fig. 62 Orthotopic transplantation; left limb bud to left side (*hom.dv.*), rotated slightly less than 180° (*i.e.* P to A in fig. 60). Exp. I. E. 86. The transplanted limb is primarily a left, having reached its normal posture by rotation; second (*2′*) and third (*3′*) digits, reduplicated. × 10.

Since these differences can scarcely be accounted for on the ground of different degree of rotation of the limb buds at operation (p. 40), it would seem that the few mesoderm cells remaining in the wound bed must have exerted some influence upon the developing limb. This does not mean that the limbs which do develop in such cases arise solely by a process of regeneration from the host. In fact, the rate of development, which is only slightly retarded below the normal, precludes such an interpretation. What probably does take place is an intermingling of cells from the host and the graft, with the result that the former, acting in the same sense as the environment with which they are in harmonic relation, counteract the tendency of the inverted elements to reverse their asymmetry. This was, however not shown to the same degree in the corresponding experiments with superposed limbs (p. 65).

9. Heteropleural transplantations, dorsodorsal orientation. Forty-nine cases were operated upon in this way and thirty-one lived long enough to yield definite results (table 2). By far the largest number of these (twenty-five) developed reduplications of one kind or other. Five cases gave rise to limbs with reversed asymmetry, i.e., to limbs which developed to fit their new surroundings, though one of these was considerably underdeveloped. One yielded a somewhat imperfect non-reversed limb and four were rudimentary. These results seem altogether divergent from the corresponding heterotopic transplantations. An examination of them shows, however, that fundamentally they accord with the latter, complete agreement being modified, by a second factor, which may suppress the original bud in favor of the reduplicating member. The normal environment of the transplanted bud and the concomitant normal functioning seem to facilitate this transformation. Moreover, there is no hard and fast line between the different results just enumerated, and the individual cases may be taken as forming a series, beginning with the single non-reversed and ending with the single reversed limb. The reduplications are intermediate. They will be considered in this order.



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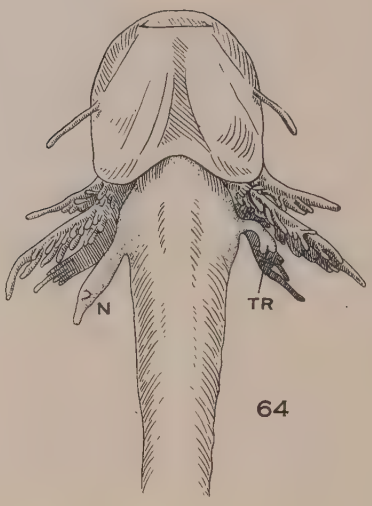

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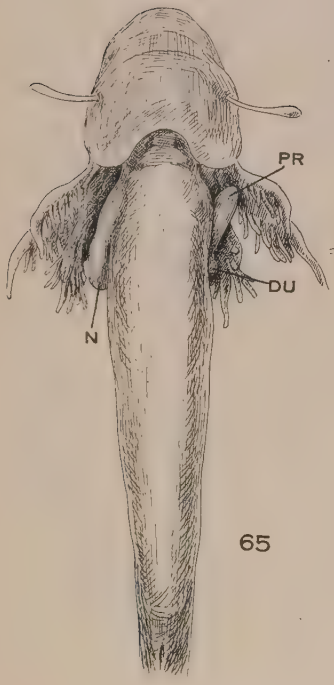

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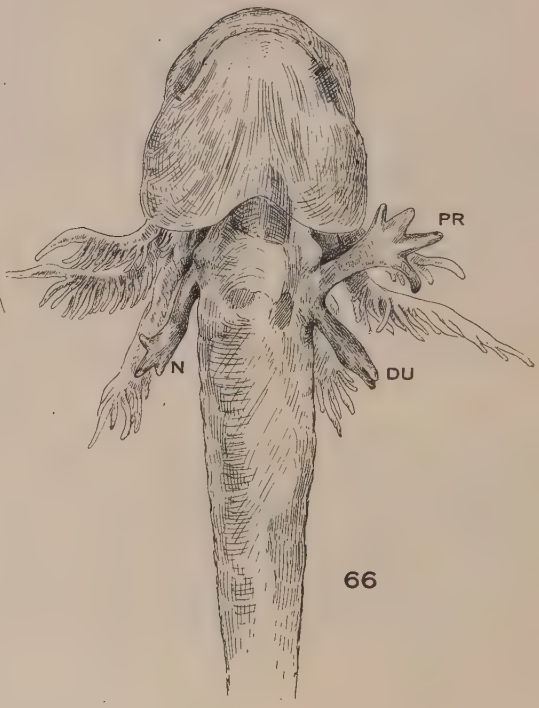

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The history of the case in which a limb of original prospective asymmetry developed[51] is given on page 126. In this individual the limb bud, as it began to grow, pointed anteriorly (fig. 63), and continued to grow in that direction. Though it remained small and imperfect (fig. 64), it is clearly a right limb on the left side (not reversed).

The cases which formed reduplications began their development in the same manner. The first direction of pointing is recorded as anterior in nine cases, anterodorsal in eight, and anterolateral in five. Three are described as pointing dorsally and one laterally. Thus these limbs all show in greater or less degree the initial effect of their original growth tendency. Growth of the bud continues then for some days in a general anterior direction, but sooner or later a reduplicating bud appears, usually at the posterior border of the original bud, and this grows in most cases into an appendage equal to or exceeding the original in size. If the reduplicating bud does not appear until late, then the original one may attain considerable size and remain, for some time at least, the principal member (figs. 65 and 66). If it appears earlier, but not until the original bud has a good start, then the two members may remain of almost equal size (figs. 67 to 71). In other cases, where the reduplicating bud begins to grow early, it soon gains the upper hand, and the original may be reduced to an atrophic or rudimentary limb (figs. 72 to 74). This condition leads over to the single reversed appendage in which the original bud is reduced to a spur or nodule

Figs. 63 and 64 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 87. Resulting limb, though defective, is reversed. *N*, normal right limb; *TR*, transplanted limb. × 10.

Fig. 63 Dorsal view, seven days after operation.

Fig. 64 Ventral view of specimen preserved sixteen days after operation.

Figs. 65 and 66 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 96. Resulting limb reduplicated.

Fig. 65 Ventral view, ten days after operation; primary limb (*PR*) points into gills; reduplicating bud (*DU*), just appearing. × 10.

Fig. 66 Ventral view, nineteen days after operation; primary member (*PR*) shows evidence of reduplication of hand; reduplicating member (*DU*) is in approximately normal position. × 10.

[51] R. E. 87.

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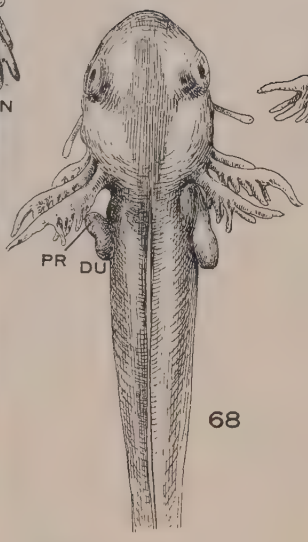

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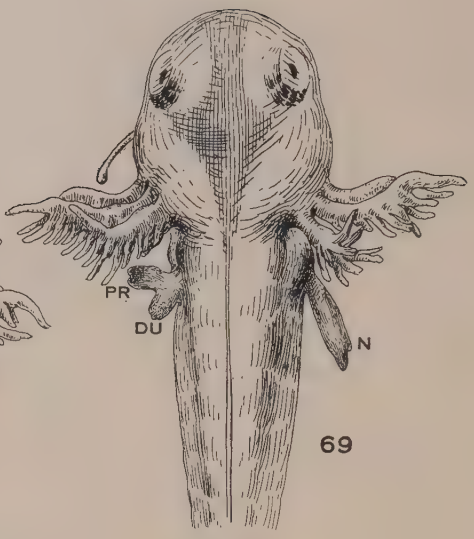

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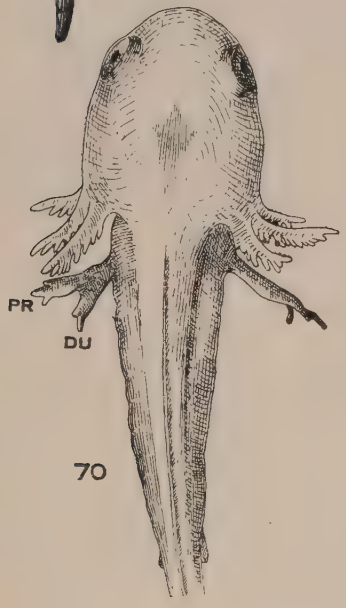

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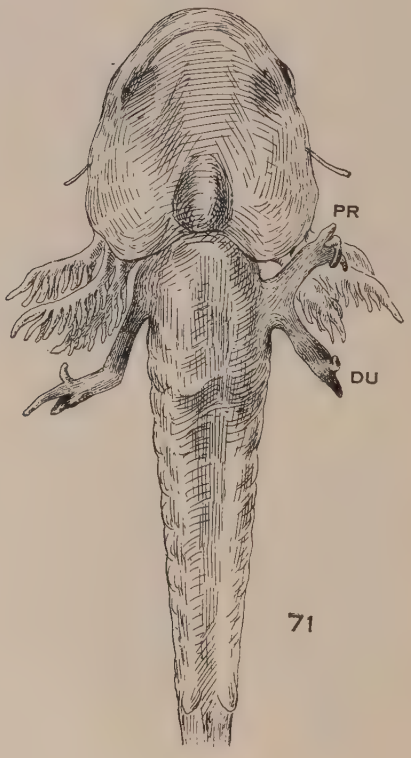

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(p. 49). The reduplicating limb is, of course, mirrored from the original and hence corresponds to the side of the body on which it is grafted.

Being placed in the position of the normal limb, the reduplication is favorably situated with regard to blood and nerve supply, and it is probably on this account that so many of them develop into functional appendages. In this respect the experiments of this group differ considerably from the preceding (homopleural inverted), where there is a greater tendency for the original member to retain its predominant condition. Otherwise the course of development in the two groups is strikingly alike.

For the study of the details of reduplication, nineteen cases are available, including one case with small wound not considered in the table. Seven others were preserved at relatively early stages in order to investigate the internal processes involved. Histories of several typical cases are given in the appendix.

Seventeen of the cases conform to the main type and do not differ materially from those considered in the last section. As in the homopleural inverted limbs (p. 35), the degree and character of the reduplication vary much from case to case. In some the digits alone are doubled, and at the other extreme we find two almost entirely separate limbs. In thirteen individuals a second reduplicating limb formed on the anterior side of the original. These usually did not develop so completely as the limbs arising from the posterior buds, and the reduplication often involved only the distal part of the manus, with the digits more or less symmetrically placed. The anterior reduplications are mirrored from the ulnar or ulnopalmar surface and occasionally from the

Figs. 67 to 70 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 70. Resulting limb reduplicated. *N*, normal right limb bud; *TR*, transplanted limb bud; *PR*, primary member; *DU*, reduplicating member. × 10.

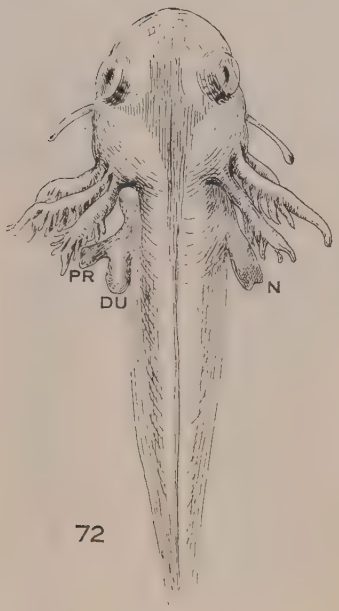
Fig.67 Dorsal view, five days after operation. The transplanted bud already gives evidence of reduplication.

Fig. 68 Dorsal view, ten days after operation. Reduplicating bud is large and in normal position.

Fig. 69 Dorsal view, sixteen days after operation.

Fig. 70 Dorsal view of specimen preserved thirty days after operation.

Fig. 71 Similar case (Exp. R. E. 71); ventral view, twenty-two days after operation. × 10.



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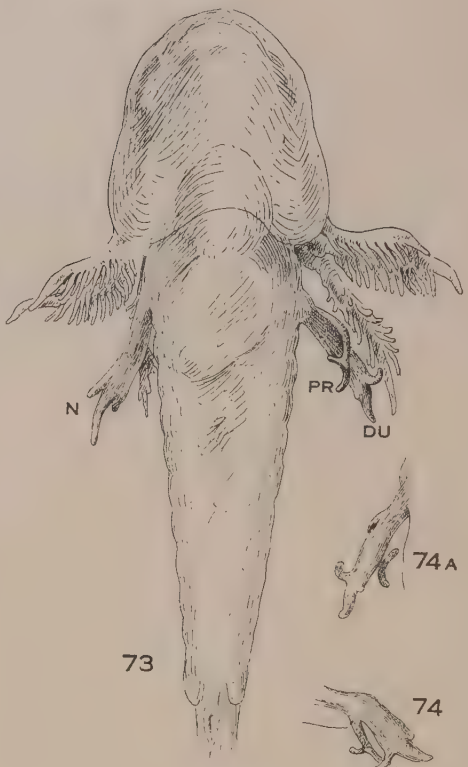

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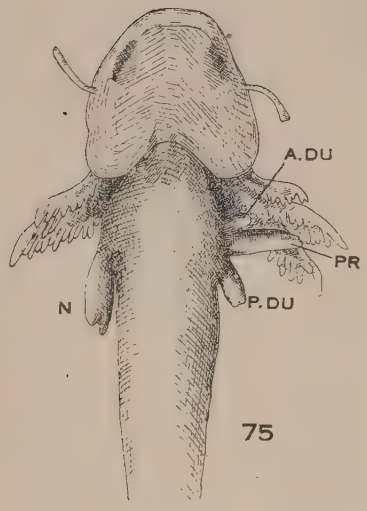

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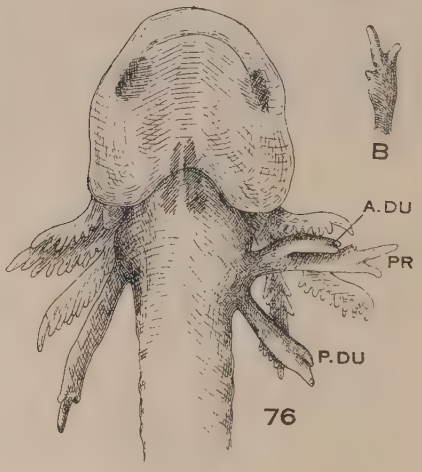

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palm. One case[52] had three almost complete separate appendages (figs. 75 and 76).

Two cases of the nineteen[53] gave rise to an anterior reduplicating bud only, which in both individuals was mirrored from the ulnopalmar surface. Owing to the position of the reduplicating bud in front of the original heteropleural limb, it could not be brought into normal posture (fig. 77).

There remain to be considered the five cases in which the asymmetry of the transplanted bud was reversed. These are of the utmost interest in showing how a secondary factor (reduplication) may so modify the result that the rules of symmetry seem not to hold. They show more than any others the necessity of having complete histories in each case, for the manner in which the end result is reached is of cardinal importance for the correct interpretation of the process. As stated above, these cases gradate into those in which duplicate limbs arise, so that the classification is somewhat arbitrary, the single-limb condition being a masked reduplication. Like the others, they begin their development with growth of the bud in an anterior direction (figs. 83 and 86). Then a posterior reduplicating bud makes its appearance, and the original bud is rapidly reduced (figs. 84 and 85 and 87 to 89) in relative importance, becoming a spur or nodule attached to the latter. The history of a typical case is given on page 128.

Figs. 72 to 74 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 74. Reduplication with atrophic primary member. *N*, normal right limb; *PR*, primary transplanted limb; *DU*, reduplicating member. × 10.

Fig. 72 Dorsal view, twelve days after operation; the primary limb already appears as an appendage of the reduplicating member.

Fig. 73 Ventral view, twenty-one days after operation.

Fig. 74 Lateral view of transplanted limb.

Fig. 74A Dorsal view of same.

Figs. 75 and 76 Orthotopic transplantation; right limb bud to left side (*het. dd.*). Exp. R. E. 133. Two almost perfect reduplicating members, one anterior (*A.DU*) and one posterior (*P.DU*) to the primary (*PR*). The relations of these limbs are just as in the diagram, fig. 4B. × 10.

Fig. 75 Ventral view, eleven days after operation.

Fig. 76 Ventral view, nineteen days after operation. *B*, lateral view of posterior reduplicating member.

[52] R. E. 133, p. 127. [53] R. E. 120 and 134.

In one individual[54] the growth in an anterior direction was well marked before the posterior reduplicating bud appeared (figs. 78 to 80). The anterior one was finally reduced to a spur, which, however, was considerably longer than in the next two cases of the series.[55] This is in reality the border-line case and might be

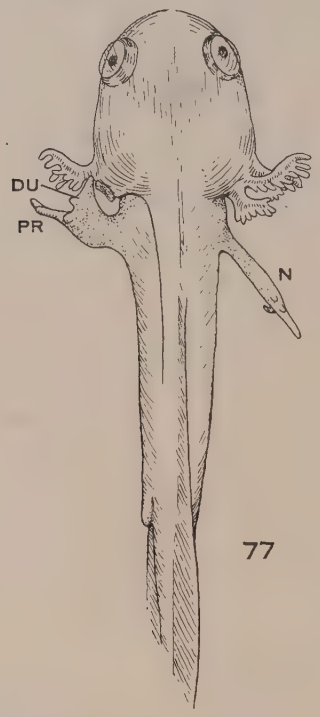


Fig. 77 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 134. The reduplicating member (*DU*) is anterior to the primary (*PR*). Preserved specimen, eighteen days after operation. × 10.

classed equally well as a reduplication. In one individual[56] the anterior bud was reduced to a slight scar, while in another[57] (figs. 90 to 92) it had only a very slight development and was soon entirely resorbed.

The cases in which the wounds were not cleaned (for the most part the earlier experiments made in 1911 and 1912) were seven-

[54] R. E. 108. [55] R. E. 77 and 69. [56] R. E. 91. [57] R. E. 95.

Figs. 78 to 82 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 108. Reduplicating member (*DU*) in normal position, developed at expense of original (*PR*), which is reduced to a long spur. *N*, normal right limb; *1* to *4*, numbers of digits. × 10.

Fig. 78 Dorsal view, six days after operation. Reduplicating bud already more massive, though less prominent, than the primary.

Fig. 79 Lateral view, six days after operation.

Fig. 80 Dorsal view, eight days after operation.

Fig. 81 Ventral view, fifteen days after operation.

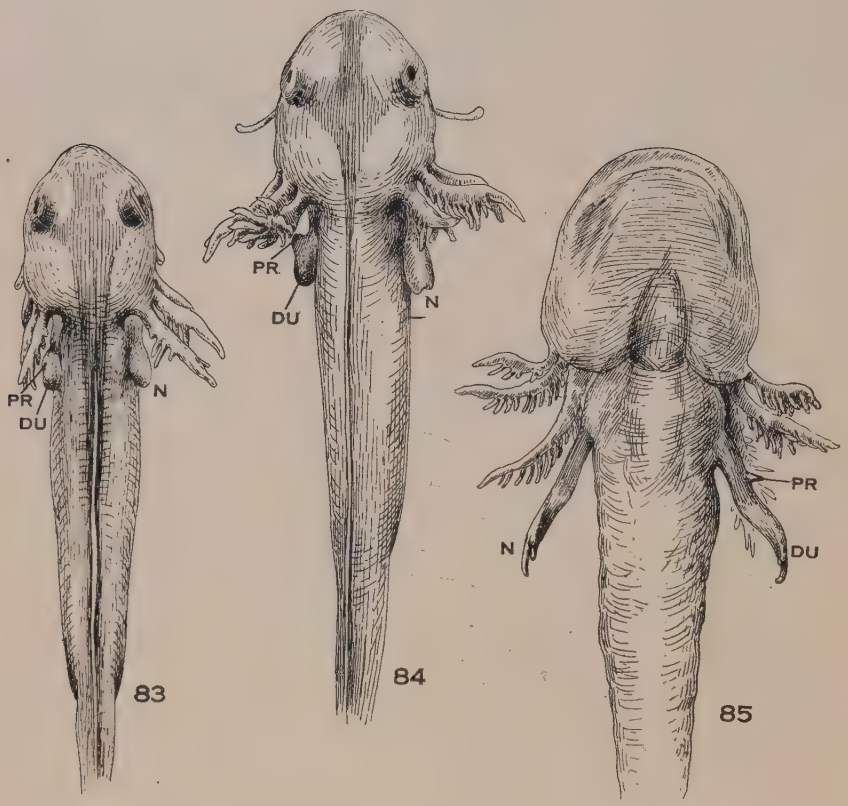
Fig. 81A Lateral view of limb.

Fig. 81B Dorsal view of same.

Fig. 82 Dorsal view, thirty-three days after operation.

Fig. 82A Ventral view of transplanted limb.

Fig. 82B Ventral view of normal right limb.



Figs. 83 to 85 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 77. Primary member (*PR*) reduced to a small spur on the reduplicating member (*DU*) which has become a normal left limb. × 10.

Fig. 83 Dorsal view, eight days after operation. Reduplication of transplanted bud already visible. × 10.

Fig. 84 Dorsal view, eleven days after operation. Reduplicating bud larger than primary.

Fig. 85 Ventral view, twenty-six days after operation.

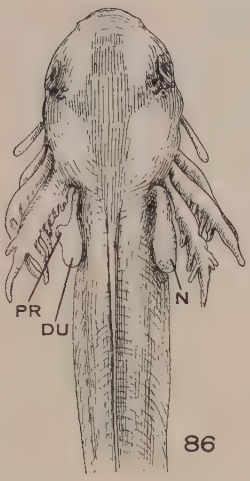
Figs. 86 to 89 Orthotopic transplantation; right limb bud to left side (*het.dd.*). Exp. R. E. 69. Primary member (*PR*) reduced to a nodule on the reduplicating one (*DU*). × 10.

Fig. 86 Dorsal view, nine days after operation.

Fig. 87 Dorsal view, twelve days after operation.

Fig. 88 Dorsal view, sixteen days after operation.

Fig. 89 Ventral view, twenty-six days after operation.



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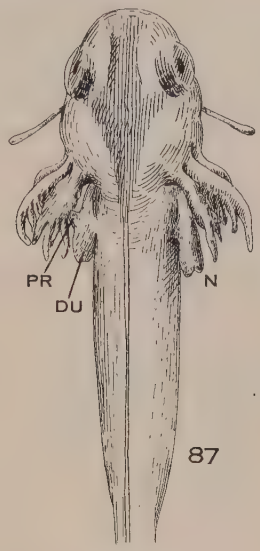

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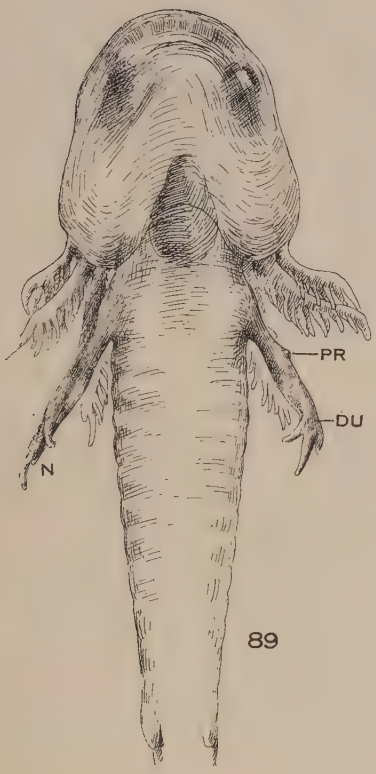

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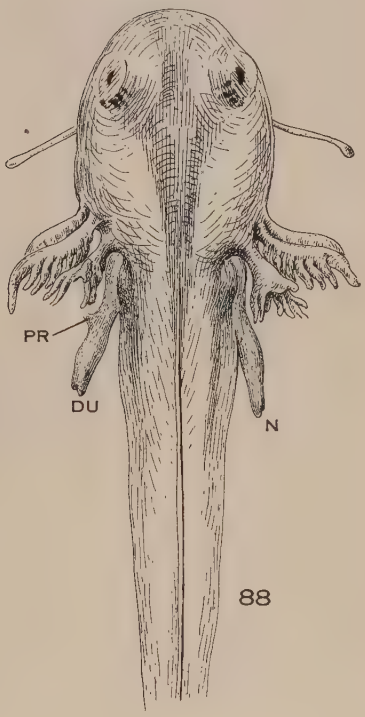

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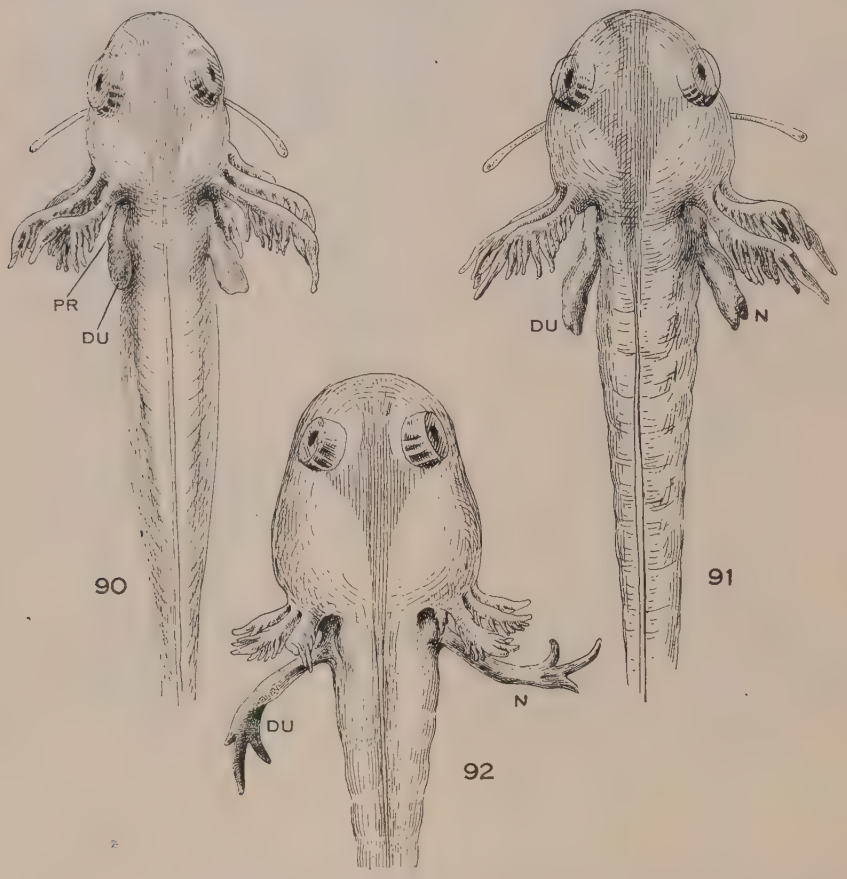
teen in number, in thirteen of which limbs developed. The distribution of these in the various groups does not show any significant differences from the cases with cleaned wounds. Eight (61.5 per cent) gave reduplications and three (23.1 per cent)



Figs. 90 to 92 Orthotopic transplantation; right limb to left side (*het.dd.*). Exp. R. E. 95. Primary bud (*PR*) entirely obliterated; the reduplicating member (*DU*) a normal left limb. *N*, normal right limb. × 10.

Fig. 90 Dorsal view, ten days after operation; the primary bud (*PR*) shows as a slight nodule.

Fig. 91 Dorsal view, thirteen days after operation.

Fig. 92 Dorsal view, thirty-three days after operation. Owing to weakness of wrist and hand extensors, the larva has difficulty in bringing its hand to normal posture.

developed into reversed limbs. In at least two of the individuals reversal was brought about by reduplication. The third is uncertain. Of the two cases (15.4 per cent) recorded as developing without reversal, only one is clear. The other died at fifteen days and was lost, so that the notes made from the living specimen could not be verified.

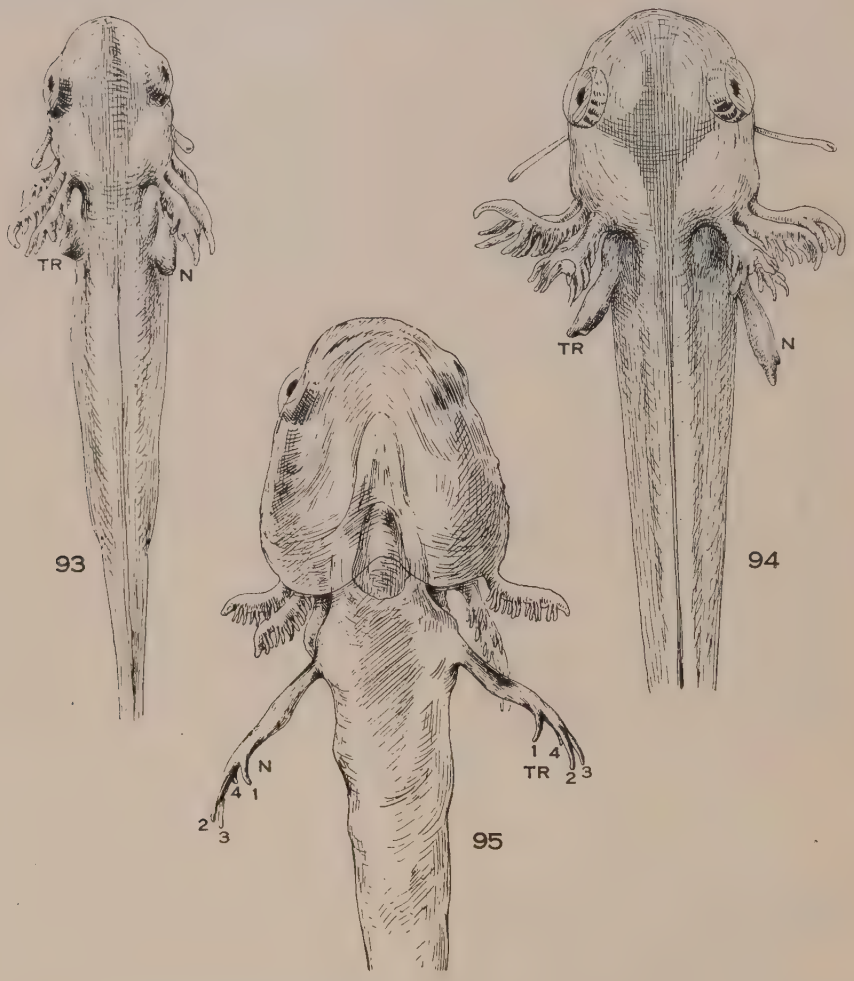
10. Heteropleural transplantations, dorsoventral orientation. Twenty-six experiments were made in this group. In five out of the twenty-three individuals that lived the transplanted tissue was resorbed, and in two others the resulting appendage was imperfect or rudimentary, so that sixteen positive cases are available. Single limbs with reversed asymmetry developed in fifteen, and only one gave rise to a duplicate structure (table 2).

This group of cases shows that from the first the transplanted limb buds behave differently from those implanted in dorsodorsal orientation. When they begin to become prominent, they point dorsoposteriorly in most cases, though sometimes more sharply dorsally and frequently more laterally than the normal bud (fig. 93). As the bud grows, it thus occupies a nearly normal position, though it may continue for some time to project more sharply to the side or more dorsally than the normal limb (figs. 94 and 96). When the third and fourth digits develop, they are, however, not formed on the ventral border of the appendage, as they would be if the original asymmetry were preserved, but they come in on the dorsal border, just as in the normal hand of the side to which they were transplanted (figs. 2, 95, 98, 99, and 102). The palm of the hand, as in the normal individual, faces the body of the larva. Besides the one case in which reduplication actually occurred, there were three others in which slight indications of doubling appeared, only to disappear later, the more ventrally lying bud soon being resorbed. Histories of typical cases are given on page 128.

In all of these cases there was some retardation of development, and in some[58] it was very marked. A somewhat greater amount of tissue is lost by disintegration when the limb is placed dorsoventrally than when placed otherwise, since the bud does not

[58] *E. g.* R. E. 90.

fit into the wound so exactly. Besides this there seems to be a time factor involved in the reversal, which would indicate that the dorsoventral axis of the limb elements is slightly differentiated, though not irreversibly so.



Figs. 93 to 95 Orthotopic transplantation; right limb to left side (*het.dv.*). Exp. R. E. 80. Transplanted limb (*TR*) becomes a normal left. *N*, normal right limb; *1* to *4*, numbers of digits.

Fig. 93 Dorsal view, six days after operation. Transplanted bud much smaller than normal.

Fig. 94 Dorsal view, fourteen days after operation.

Fig. 95 Ventral view, forty-two days after operation.

The sole case in which a double appendage resulted[59] is interesting, inasmuch as it shows that the primary bud grows into a reversed limb, while the reduplicating bud has the original asymmetry (figs. 103 to 105). This is the opposite of the result obtained when the bud is implanted in dorsodorsal orientation. (History on p. 129.)

In the experiments with wounds not cleaned the proportion of reduplications is considerably larger—seven out of fifteen, or 46.7 per cent, as against one case in sixteen in the clean-wound group. There were eight cases (53.3 per cent) in which normal limbs with reversal of asymmetry developed, as against fifteen (94 per cent) in the case of the clean-wound experiments.

11. The shoulder-girdle in orthotopic transplantations. The above account has dealt only with the external features of the limb. The shoulder-girdle is likewise of interest.

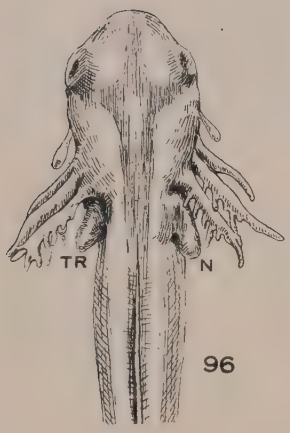
As the heterotopic transplantations show, a small portion of the girdle surrounding the glenoid cavity always develops in connection with the grafted limb. After extirpation of the limb bud, however, the outlying regions of the girdle, including the suprascapula and portions of the procoracoid and coracoid, develop from cells that are left in the host.[60] It was to have been expected, therefore, that relations of harmony or disharmony would manifest themselves in the shoulder-girdle in orthotopic grafts. Study of serial sections of some of the cases shows that this is usually the case. Twelve individuals, belonging to three different groups have been examined in this way.

The three harmonic grafts (*het.dv*) all show girdles that are normal, except that they are somewhat underdeveloped. There has obviously been a union of host and graft tissues to form a normal whole, in spite of the fact that the transplanted bud was from the opposite side of the body.

The nine disharmonic grafts all show some form of irregularity, and in nearly all cases there is some sort of double girdle with reversal of the part that is derived from the graft. The condition of the girdle is complicated by the reduplication of the free

[59] R. E. 93.

[60] Cf. Detwiler, '18, p. 503, and Harrison, '18, p. 429.



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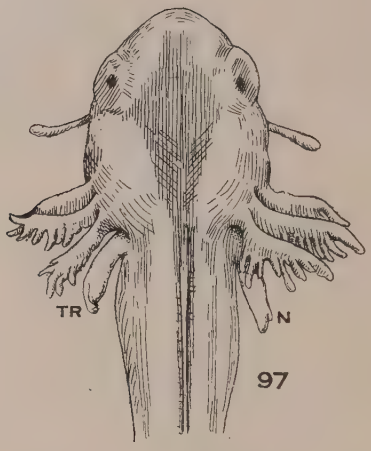

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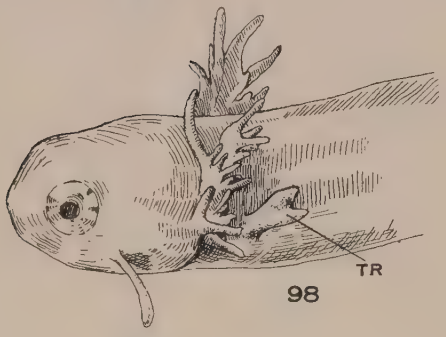

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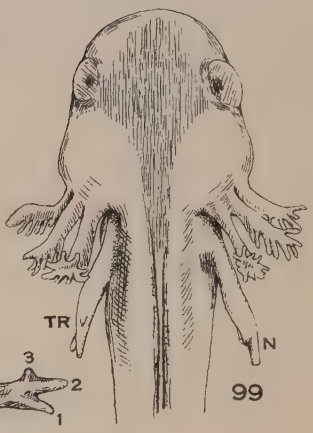

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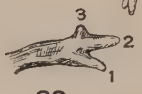

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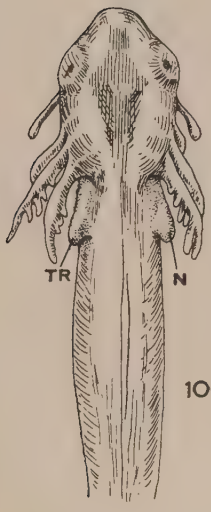

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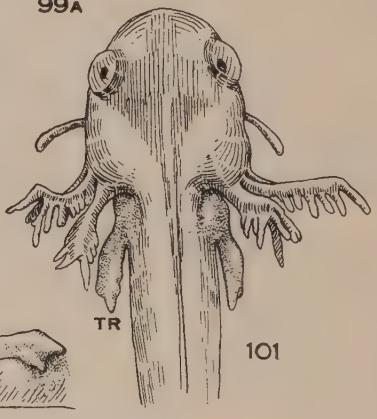

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limb which takes place in most cases (table 2). It is more readily understood in the two cases in which a single limb of opposite asymmetry is present.

In the first of these,[61] in which a limb bud from the same side of the body was implanted in inverted position (p. 32, figs. 39 to 41), there are two entirely distinct shoulder-girdles. The anterior one has no connection with the other and is undoubtedly derived from the host, having the characteristics of girdles which develop after extirpation of the limb bud. The scapula and suprascapula are already joined in cartilaginous union with the procoracoid, but the coracoid is connected with the latter by ligament only. The girdle belonging to the transplanted limb is mainly posterior to the other, though there is some overlapping. It is large to have developed from a transplanted bud, but it has the characteristics of such. There is a distinct procoracoid process as well as a large coracoid, both of which project posteriorly from the glenoid cavity. This girdle is clearly reversed, as is the transplanted limb which is connected with it.

The other single disharmonic limb is the one developed from a bud taken from the opposite side of the body.[62] The limb itself is atrophic (fig. 64). The girdle is double, but the ventral parts of the two members are fused. The suprascapula, which is single and belongs to the host, is not connected with the rest. The

Figs. 96 to 99 Orthotopic transplantation; right limb to left side (*het.dv.*). Exp. R. E. 107.

Fig. 96 Dorsal view, eight days after operation. Transplanted bud (*TR*) smaller than normal (*N*) and more pointed.

Fig. 97 Dorsal view, thirteen days after operation.

Fig. 98 Lateral view, thirteen days after operation.

Fig. 99 Dorsal view, nineteen days after operation.

Fig. 99A Lateral view of transplanted limb. *1* to *3*, numbers of digits.

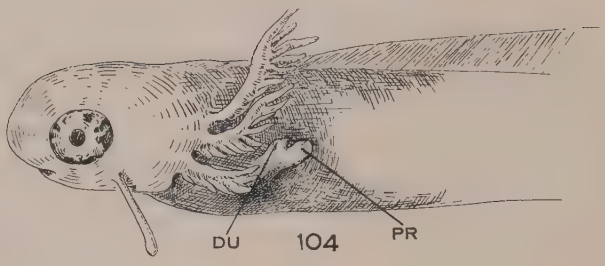
Figs. 100 to 102 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 116. Transplanted limb (*TR*) becomes a normal left. × 10.

Fig. 100 Dorsal view, seven days after operation. Transplanted bud only slightly smaller than normal.

Fig. 101 Dorsal view, thirteen days after operation.

Fig. 102 Lateral view, same age.

[61] I. E. 64. [62] R. E. 87.



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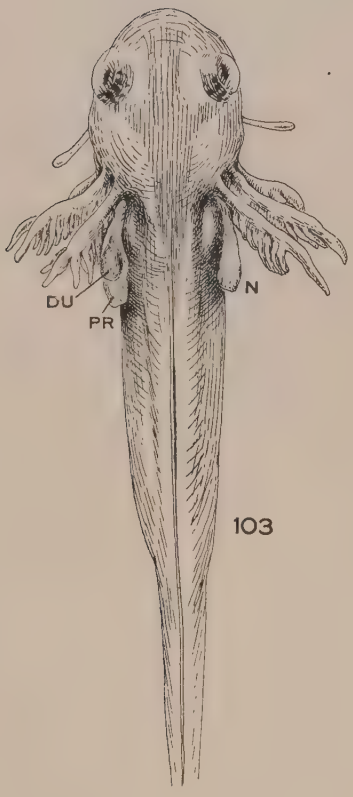

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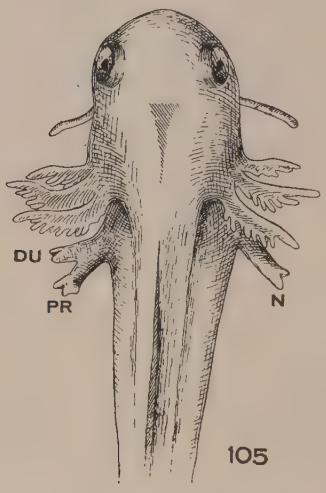

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Figs. 103 to 105 Orthotopic transplantation; right limb bud to left side (*het.dv.*). Exp. R. E. 93. Reduplication, the primary (*PR*) member being a left (reversed). *DU*, reduplicating member. × 10.

Fig. 103 Dorsal view, nine days after operation.

Fig. 104 Lateral view, same age.

Fig. 105 Dorsal view, preserved specimen, age fifteen days.

ventral part, which forms the glenoid cavity, is in fore and aft symmetry, with a coracoid and procoracoid process pointing in each direction. The posterior half of this cartilage has almost certainly developed in connection with the grafted limb and is reversed, while the anterior half is derived from the host.

In the disharmonic cases which have reduplicated limbs, the shoulder-girdles are on the whole less regular, owing to the complex articulations of the double appendages. One of them[63] (*hom.dv*) is, however, similar to the one first described in having two entirely separate girdles, one derived from the host and one from the graft. The suprascapula, procoracoid, and coracoid of the former are separate chondrifications, situated directly opposite the corresponding parts of the normal limb. The girdle of the transplanted limb has a broad flat glenoid cavity for articulation with the massive humerus. There is a large coracoid running ventrally from the joint, though without any very well-marked procoracoid. This girdle is placed some distance posterior to that of the host. Another of these cases[64] (*hom.dv*) is more like the second case described above, inasmuch as the dorsal element (suprascapula) is separate, while the two coracoids (from host and graft, respectively) are fused. The procoracoid of the host is a separate cartilage in this case. Two other cases[65] are of the same general type with fused coracoids, though they are rather too young to show all characteristics. Again, two others[66] have two scapulae with coracoids fused. There is only one case[67] that shows in sections practically no sign of doubling of the girdle, though even in this the coracoid region is thicker than normal and the glenoid cavity is large in correspondence with the more massive humerus.

To sum up: The shoulder-girdle in orthotopically grafted limbs is derived in part from the host and in part from the transplanted tissue. The former portion retains its normal asym-

[63] I. E. 81.
[64] I. E. 93.
[65] I. E. 68 (*hom. dv.*) and R. E. 129 (*het dd.*).
[66] R. E. 77 (*het. dv.*) and R. E. 96 (*het. dv.*).
[67] I. E. 60 (*hom. dv.*).

metry, while the latter behaves in accordance with the rules governing the asymmetry of transplanted limbs. In the disharmonic combinations the portions of the girdles derived, respectively, from the two sources may fuse together or may remain entirely separate. In the harmonic combinations they unite to form a single normal girdle.

12. Summary of the results of orthotopic transplantations. The orthotopic transplantations develop according to the same rules as the heterotopic. In the homopleural dorsodorsal and the heteropleural dorsoventral groups rules 1 and 2 (p. 4) are very closely followed. In the former the limb buds, being right side up, retain their normal asymmetry; and in the latter, being upside down, they reverse it. In both groups this results in limbs which correspond to the side on which they are implanted (harmonic combinations).

In the other two groups the primary single limbs which develop do not correspond to the organic environment, since the homopleural graft, when placed upside down, becomes reversed, and the heteropleural graft right side up retains its original prospective asymmetry. In these combinations, which have been called disharmonic, single limbs are, however, the exception. It is here that rule 3 comes into play. Reduplications occurred in 71.1 per cent of the cases in the homopleural dorsoventral group and in 80.6 in the heteropleural dorsodorsal. The former includes only one case of single limb reversed. In this class are also five cases of reversed single limbs, which are fundamentally the same as reduplications, the original limb having been suppressed or resorbed. The disharmonic relation thus augments immensely the tendency to reduplicate. In the case of the heterotopic grafts, on the contrary, the greater proportion of reduplications occurs in the harmonic combinations. This curious fact will be discussed below (p. 107). The ten cases of non-reversed single limbs which resulted from homopleural inverted buds are, as already pointed out, exceptional in that the limb regained its normal posture gradually during development by rotation at the base.

C. Superposed limb buds

In the preceding study of transplanted limbs certain experiments were described, which showed that the mesoderm from two limb buds, when fused together, would develop into a single normal limb. At first larger than normal, the size of such a limb is soon regulated. In the former communication only those experiments were considered in which the orientation of the superposed bud was normal (*hom.dd*). The effect of the orientation of the graft will now be taken up.

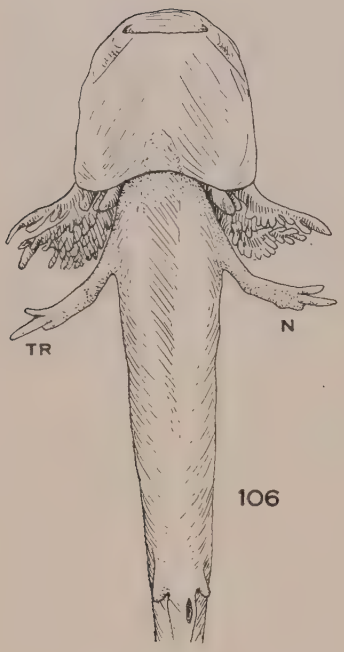
TABLE 3

Superposed limbs. Summary of results

OPERATION	NUMBER OF EXPERIMENTS		NORMAL SINGLE LIMBS		NORMAL SINGLE WITH REDUCED REDUPLICATION		REDUPLICATED	
	Total	Positive	Number	Per cent	Number	Per cent	Number	Per cent
Hom. dd	5	5	5	100	0	00	0	00
Hom. dv	5	5	1	20	0	00	4	80
Het. dd	6	5	0	00	1	20	4	80
Het. dv	9	5	5	100	0	00	0	00
Total	25	20	11	55	1	5	8	40

The experiments are summarized in table 3. There were twenty-five operations, of which twenty are available for the analysis. Two of the combinations, the ones which the ordinary transplantations have shown to be harmonic (homopleural dorsodorsal and heteropleural dorsoventral) yielded only normal appendages (ten cases). The two disharmonic combinations (homopleural dorsoventral and heteropleural dorsodorsal) yielded reduplications in nine cases out of ten. One case, in which one member of the duplicate limb was reduced to a spur, is included among the reduplications.

13. Homopleural transplantations, dorsodorsal orientation. In this group development went forward with a minimum of disturbance. The only abnormal feature to note is the large size of the double bud in certain individuals. In several of the cases



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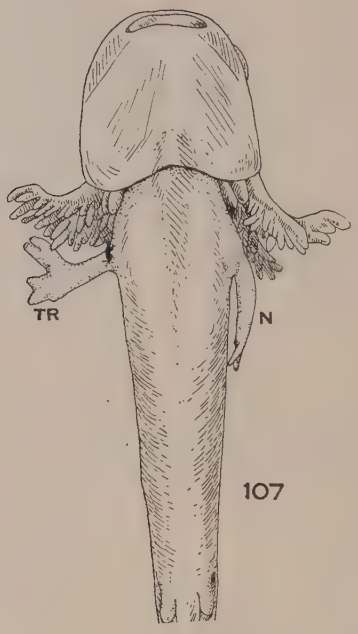

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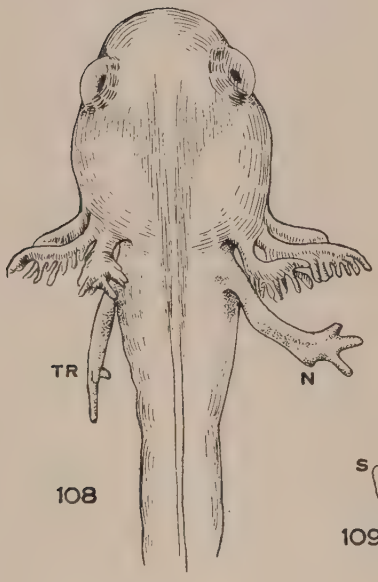

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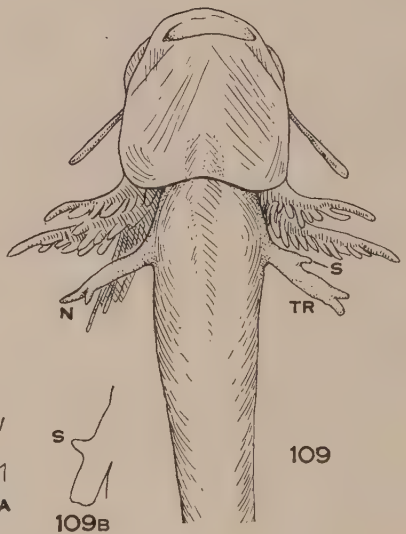

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109B

the difference from normal size persisted for twelve or more days,[68] gradually diminishing during that period (fig. 106). In others the difference was less marked, though in all some difference in favor of the limb on the operated side was noted.

14. Homopleural transplantations, dorsoventral orientation. Four out of the five cases in this group gave rise to reduplications in the grafted limb.

The reduplications vary. One[69] is a typical case of radial mirroring of the lower part of the forearm and hand (fig. 107). Another[70] is similar, except that the anterior member is itself reduplicated, the hand being a nearly symmetrical complex with four digits. These two individuals are in every respect like those cases of reduplication resulting from simple inverted limb buds, in which the primary member is reversed and is accompanied by a non-reversed twin which takes up the normal position. In two other cases[71] the reduplication is less and is of a character not necessarily attributable to disharmonic combination, though there is nothing to indicate that it is not due to such a cause. Principally the digits are involved (fig. 108).

In the remaining case[72] a normal limb developed. This one and possibly also the two foregoing are analogous to those cases of simple transplantation in which the inverted limb bud develops into a normal limb without reversal by means of rotation (p. 40).

Fig. 106 Superposed limb bud; right limb bud to right side, normal position (*hom.dd.*). Exp. S. E. 3. Normal limb (*TR*) on operated side. *N*, normal left limb. Preserved specimen, ventral view, eighteen days after operation. × 10.

Fig. 107 Superposed limb bud; right limb to right side inverted (*hom.dv.*). Exp. S. E. 18. Reduplicated appendage (*TR*) on operated side. Preserved specimen, ventral view, eighteen days after operation. × 10.

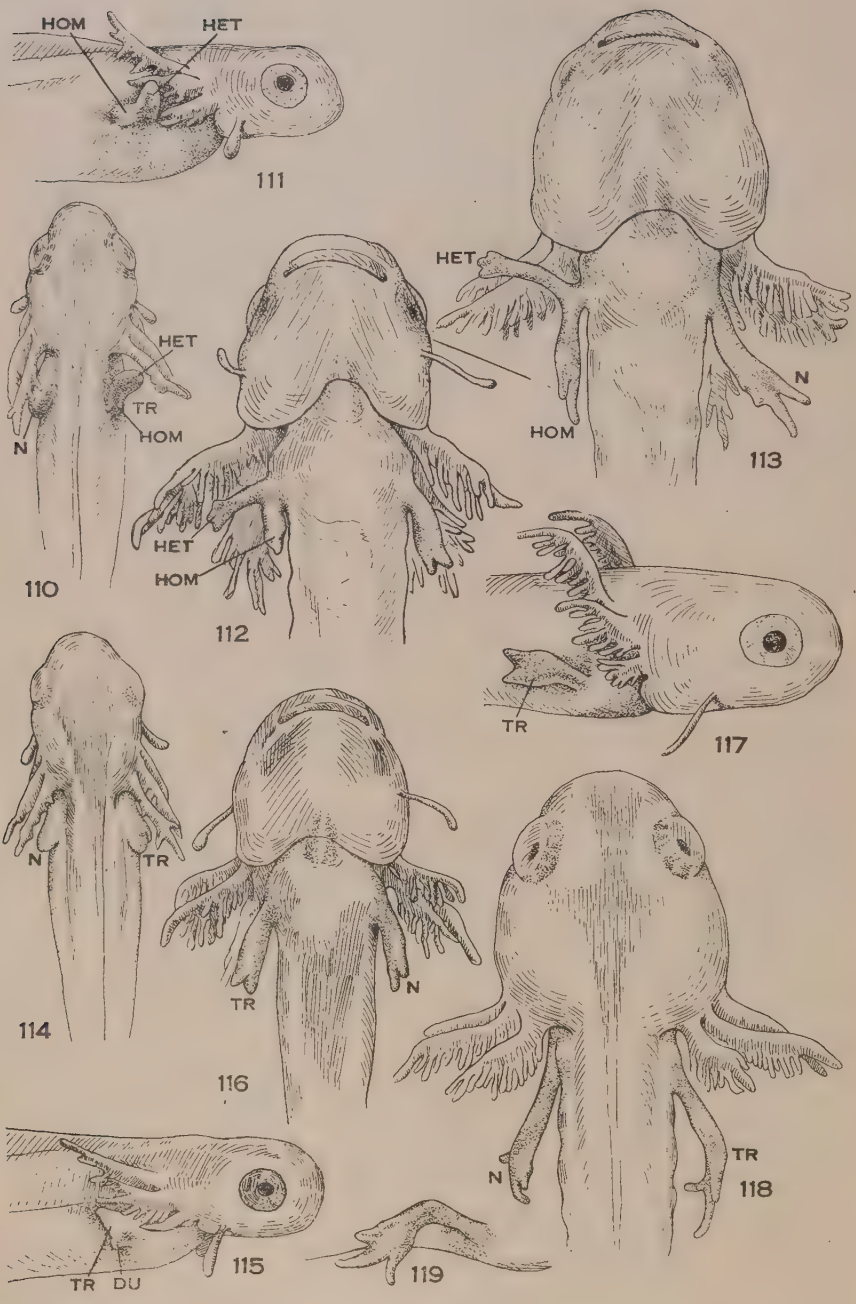
Fig. 108 Superposed limb bud; right limb bud to right side inverted (*hom.dv.*) Exp. S. E. 9. Operated limb (*TR*) normal except for reduplication of second digit. × 10.

Fig. 109 Superposed limb bud; right limb bud to left side (*het.dd.*). Exp. S. E. 6. Reduplication with heteropleural member reduced to spur (*S*). × 10.

Fig. 109A Outline of limb bud from above. Five days after operation (free-hand sketch).

Fig. 109B Same, eleven days after (free-hand sketch).

[68] S. E. 3. [69] S. E. 18. [70] S. E. 2. [71] S. E. 9 and 14. [72] S. E. 10.


HOM
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114
TR
N
116
N
TR
118
TR
DU
115
119

15. Heteropleural transplantations, dorsodorsal orientation. In this group the results of five experiments all fall clearly within the same category. A normal appendage in approximately normal position developed, and this had a reduplicating member attached to its radial border. The differences between the cases consist in the extent of reduplication and the degree of development attained by the disharmonic (anterior) member. The most extreme cases are one[73] in which the heteropleural member is reduced to a spur (fig. 109), and one[74] in which there is almost complete doubling from just below the shoulder down, the heteropleural member being, however, atrophic (figs. 110 to 113). Two of the three remaining cases[75] are very similar to one another, the anterior member with two digits arising from near the elbow. In the third individual the hand is reduplicated[76] externally, and the whole arm is somewhat shorter and thicker, indicating some degree of internal reduplication.

16. Heteropleural transplantations, dorsoventral orientation. The five cases in this class all gave normal limbs (figs. 117 and 118). Three of them showed slight indication of doubling (figs. 114 and 115) in the early stages of development (four or five days after operation), but the more ventral-lying prominence had disappeared at the time of the next observation in each individual

Figs. 110 to 113 Superposed limb bud; left to right side (*het.dd.*). Exp. S. E. 12. Reduplication. *N*, normal left limb; *TR*, operated limb; *HET*, heteropleural member; *HOM*, homopleural member.

Fig. 110 Dorsal view, five days after operation.

Fig. 111 Lateral view, same age.

Fig. 112 Ventral view, ten days after operation.

Fig. 113 Ventral view, seventeen days after operation.

Figs. 114 to 119 Superposed limb bud; left to right side (*het.dv.*). Exp. S. E. 11. Operated limb (*TR*) of large size; *N*, normal unoperated limb. × 10.

Fig. 114 Dorsal view, five days after operation.

Fig. 115 Lateral view, same age. A distinct nodule or bud (*DU*) on ventral border of limb was soon afterward resorbed.

Fig. 116 Ventral view, ten days after operation.

Fig. 117 Lateral view, same age.

Fig. 118 Dorsal view, seventeen days after operation.

Fig. 119 Lateral view of limb, same age.

[73] S. E. 6. [74] S. E. 12. [75] S. E. 8 and 21. [76] S. E. 15.

(figs. 116 and 117). A sixth case[77] is inconclusive owing to general weakness of embryo, but it is not inconsistent with the results in the other cases. A typical history is given in the appendix (p. 131).

Though more than the normal amount of material is present in the composite limb bud, in two of the cases of this group the developing limb is recorded at first as slightly smaller than the normal. In one case no difference in size was noted, while in two the limb on the operated side is noted as larger. In the other harmonic combination (homopleural dorsodorsal) all cases showed the limb on the operated side to be somewhat larger in size.

17. Discussion of experiments with superposed limb buds. The principal differences between these experiments and those of simple transplantation are that in the former the tissue available for the formation of the limb is approximately double in amount, and there is a mixture of tissues having two different orientations except in the one group, homopleural dorsodorsal. In the harmonic combinations the amount of tissue is so regulated that after a time size-differences disappear. The amount of tissue is, moreover, never quite double that of the normal limb because of the material lost by the operation and of the general retardation of growth due to the same cause. In the case of the heteropleural dorsoventral grafts, which are classed as harmonic, some readjustment must be necessary, as shown by the amount of retardation.

In all of the disharmonic combinations there is a mixture of tissues differently oriented and with different prospective meaning as regards the particular asymmetry of the future limb. The twin limbs that arise are, therefore, not necessarily due to reduplication by budding, as they must be in the simple transplantations, but probably in part at least to the circumstance that one of the pair develops out of the original limb bud, while the other is from the transplanted tissue.

[77] S. E. 16.

D. Transplantation of half buds

Partly as a further test of the question of equipotentiality and partly to study more thoroughly the effect of harmonic and disharmonic combinations, a series of experiments with half limb buds was instituted. Instead of removing the whole circular disc comprising the limb rudiment, a semicircular piece was cut out, the wound bed carefully cleaned, and the removed portion replaced by a piece of similar size and shape from another limb bud. Considering only vertical and horizontal halves and replacing vertical only with vertical and horizontal only with horizontal, there are sixteen different experiments possible, which have been numbered in the diagram (fig. 120) from 1 to 16.

There are five different pairs of attributes, which appear as alternatives in the operations. Thus the transplanted half bud is either—1) homopleural (*hom.*) or heteropleural (*het.*); 2) upright (*dd.*) or inverted (*dv.*); 3) homogeneous (*homogen.*) or heterogeneous (*heterogen.*); 4) vertical (*vert.*) or horizontal (*horiz.*); 5) anterior (*ant.*), dorsal (*dors.*) or posterior (*post.*), ventral (*vent.*).

This aggregation would consist of 2^5 or thirty-two classes, were it possible to combine the attributes of operation independently without restriction, as would be the case were the pieces rectangular. Since, however, they are semicircular they fit in only half the cases, and the total is therefore reduced to sixteen. All of the possible experiments have been performed.

If both halves of the disc are considered movable, further possibilities open up. There would then be thirty-two different combinations, which, however, in eight cases would be practically identical with the experiments where the whole disc is transplanted. None of these experiments have been performed, since the technical difficulties would be at least doubled, and, as far as the study of either of the questions at issue is concerned, they would offer no advantage over those in which only one half of the bud is transplanted. Again, were the limb disc homogeneous, either or both halves could be turned inside out and then one hundred and twenty-eight different combinations would be possible. These are precluded, as in the case of the whole discs, by the

impossibility of grafting successfully pieces with the mesoderm turned toward the outside and by the difficulty of handling pieces of mesoderm free from ectoderm without disturbing their arrangement. Perhaps we may consider ourselves fortunate in being subject to such restrictions.

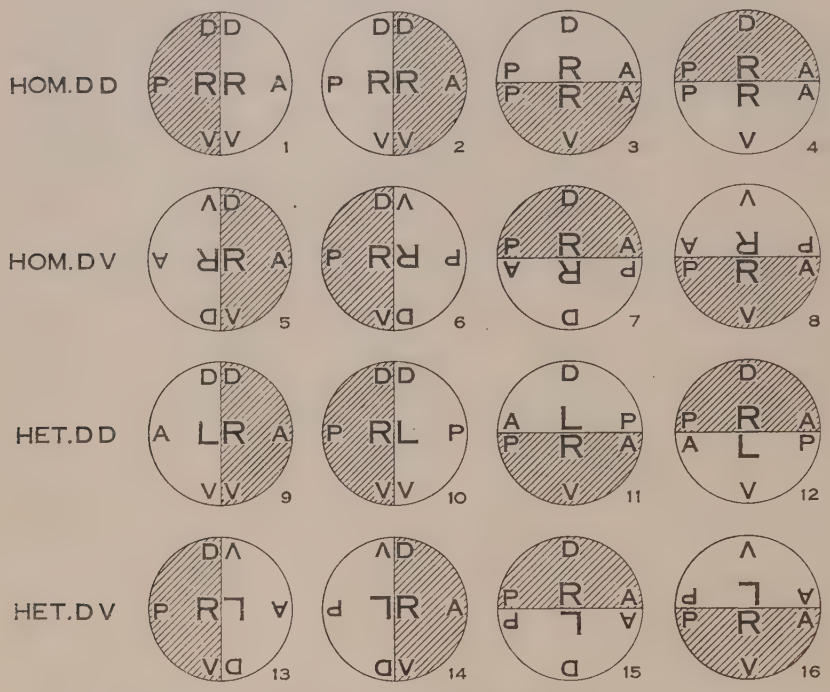


Fig. 120 Diagram showing the sixteen possible combinations (1 to 16) obtainable by transplanting half limb buds. The shaded area signifies the stationary half, the clear area the transplanted half. *R*, right; *L*, left; *D*, Dorsal; *V*, ventral; *A*, anterior; *P*, posterior. The operations are represented as on the right side of the embryo.

Returning to the experiments actually carried out (fig. 120), we find that four of them consist merely in replacing the excised piece with another of exactly the same kind in normal orientation. These serve, therefore, as controls for testing the effect of the operation as such on the further course of development. It is also seen that half of the combinations are harmonic and half disharmonic (p. 8). Half are of course homogeneous or com-

posed of two similar halves, while the other half are heterogeneous. Of the former, six belong to the disharmonic group and only two to the harmonic, while of the latter the reverse is the case, a circumstance that affects the proportionate results of the experiments.

The effect of removal of the various halves of the limb rudiment has already been described (Harrison, '18). As shown by such experiments, any half of the limb bud can give rise to a whole limb, though quantitatively the material is eccentrically distributed, there being more limb-forming tissue in the dorsal and anterior halves than in the ventral and posterior halves, respectively. Accordingly, four of the homogeneous combinations would have somewhat less than the normal amount of tissue, while four would have a little more. In the later experiments an attempt was made to compensate for this by not cutting the area exactly in half.

Owing to the large number of combinations in the experiments, it has not been possible to perform a sufficient number of each, for accurate statistical treatment. The number is sufficient, however, to compare the more comprehensive groups; for instance the homogeneous with the heterogeneous and the harmonic with the disharmonic.

Seventy-nine operations were done, sixty-eight healing successfully. Badly defective limbs developed in but four cases, so that sixty-four remain for the purpose of the analysis. These experiments are summarized in table 4.

From the results of transplanted whole limbs we should expect the following to take place; the harmonic combinations should give rise to simple normal limbs, the disharmonic to reduplications. The homogeneity or heterogeneity of the combination should not be expected to make any difference in view of the other tests of the equipotentiality of the system. These expectations were in the main realized, probably in fifty-five out of the sixty-four cases (85.9 per cent) (table 7). There are, however, sources of confusion, which in certain cases make several interpretations possible, and which for this and other reasons must not be overlooked. For example, it is known from experiments with whole

limb buds, that a normal limb may arise from a disharmonic combination by the suppression of the original bud or by its reduction to a mere excrescence on the reduplicating member, which latter may develop into a normal limb. Eight of the ten normal cases which would otherwise appear anomalous may certainly be thus explained, and possibly the remaining two. It has been found

TABLE 4

Transplantation of half limb buds. Summary of results of actual experiments

OPERATION						RESULTING LIMB				
Number	Side of origin of graft	Orientation	Composition	Direction of halving	Designation of transplanted half	Normal	Normal by resorption	Reduplicated	Abortive	Dead
1	hom.	dd	heterogen.	vertical	ant.	2	0	0	0	1
2	hom.	dd	heterogen.	vertical	post.	2	0	0	0	1
3	hom.	dd	heterogen.	horiz.	dors.	2	0	0	0	0
4	hom.	dd	heterogen.	horiz.	vent.	2	0	0	0	0
5	hom.	dv	homogen..	vertical	ant.	0	2	2	0	1
6	hom.	dv	homogen.	vertical	post.	0	0	5[2]	0	2
7	hom.	dv	homogen.	horiz.	dors.	0	2	2	0	0
8	hom.	dv	homogen.	horiz.	vent.	0	0	4	1	0
9	het.	dd	homogen.	vertical	ant.	2	2	0	3	1
10	het.	dd	homogen.	vertical	post.	0	1	3[1]	0	0
11	het.	dd	heterogen.	horiz.	dors.	0	0	5[1]	0	1
12	het.	dd	heterogen.	horiz.	vent.	0	0	4	0	1
13	het.	dv	heterogen.	vertical	ant.	4	0	1	0	1
14	het.	dv	heterogen.	vertical	post.	4	0	0	0	2
15	het.	dv	homogen.	horiz.	dors.	5	0	2	0	0
16	het.	dv	homogen.	horiz.	vent.	6	0	0	0	1
Total number of cases, 79; positive cases, 64....						29	7	28	4	11

[1] Includes one case of anomalous reduplication.

[2] Includes two cases of anomalous reduplication.

also that almost any transplantation or even simple defect experiment may sometimes bring about reduplication. The three anomalous reduplications, being slight, are probably of this class. A further source of error might arise from the circumstance, that either the grafted or the stationary half may in certain cases be solely responsible for the limb that develops; for it is known, on the one hand, that any graft may be resorbed and, on the other,

that when half the disc is excised, complete suppression of development may sometimes result, probably through accidental injury to the remaining part. The result of the former contingency would be confusing, owing to the development of a normal limb in place of a reduplication.

In connection with these questions it must also be borne in mind that the cases of union of two disharmonic halves differ, with respect to the disharmony of the combination, from those in which the limb-bud has been transplanted as a whole. In the former only one half of the rudiment is involved, the other being in all cases harmonic with the surrounding tissues. We are, therefore, dealing with a rudiment that is disharmonic in itself, while in the case of the whole limb the transplanted bud is harmonic in itself, though disharmonic with respect to the organism as a whole. This might possibly give rise to some differences in the results in the two classes of experiments.

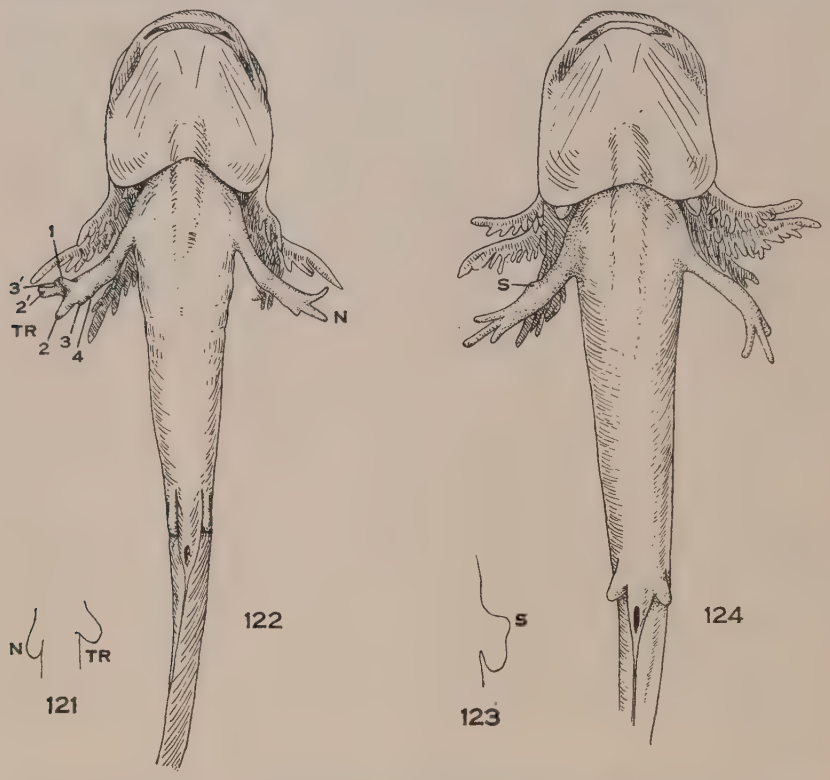
In view of these considerations, we should not expect the transplantation of half buds to give such clear-cut results as the experiments with whole ones. On the other hand, it must not be overlooked that the sources of confusion above enumerated, while accounting for nearly all of the anomalies, also render less cogent the cases which conform to the rules. Nevertheless, after taking all circumstances into consideration, it can scarcely be doubted, that the experiments with half discs do afford a valuable confirmation of the results obtained from the other experiments.

18. Homopleural transplantations, dorsodorsal orientation. The eight cases of homopleural grafts in upright orientation (*hom.dd*), two involving each half of the bud, all resulted in normal limbs, as was to have been expected, for this operation is nothing more than replacing an excised portion with one exactly similar. Only slight retardation of development is recorded in some of the cases.

19. Homopleural transplantations, dorsoventral orientation. The nineteen experiments with homopleural grafts in inverted position (*hom.dv*) resulted, in accordance with expectation, in a large number (fifteen) of duplicities[78] (figs. 121 and 122). The

[78] H. E. 29 and 31.

remaining four cases were normal. In the early stages, however, all of the latter gave evidence of reduplication (fig. 123). The limb bud, when it first appeared, showed two distinct nodules or prominences, one of which developed into a normal limb. In



Figs. 121 and 122 Transplantation of half limb bud (comb. 6, fig. 120); posterior right to anterior right (*hom.dv.*). Exp. H. E. 31. Partial reduplication of hand, mirror plane being radiodorsal. Arm and medial hand homopleural. *1* to *4*, numbers of digits of main hand; *2'*, *3'*, digits of reduplicating member.

Fig. 121 Outline of normal (*N*) and operated (*TR*) buds from above, nine days after operation (free-hand sketch).

Fig. 122 Ventral view, preserved specimen twenty-one days old. × 10.

Figs. 123 and 124 Transplantation of half limb bud (comb. 7, fig. 120); dorsal right to ventral right (*hom.dv.*). Exp. H. E. 2. Operated limb slightly thicker, reduplicating member reduced to a nodule (*S*).

Fig. 123 Outline of operated limb, dorsal view eight days after operation (free-hand sketch).

Fig. 124 Ventral view preserved specimen, twenty days old. × 10.

three cases the other prominence persisted also, at the elbow in one[79] in the form of a spur (fig. 124), and as a nodule at the shoulder in two others.[80] In the remaining case[81] all external traces of reduplication disappeared.

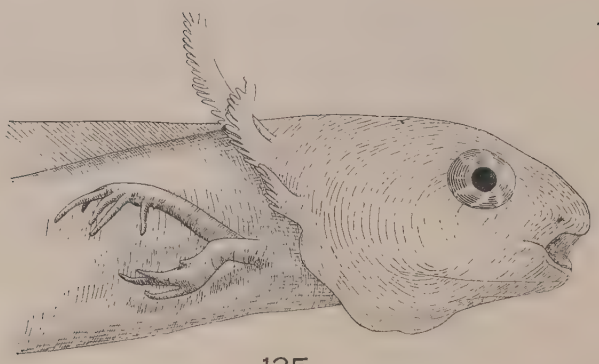
On the other hand, two of the cases of reduplication are of an anomalous nature and cannot be regarded as conforming to the rule. Both of these were experiments in which the anterior half of the limb bud was replaced by a posterior half. We should expect in such a case to find posteriorly a homopleural member developed out of the stationary portion of the bud, while anteriorly there should be a reversed limb which might itself be reduplicated. The opposite is, however, true. In both cases the anterior member is not reversed. The posterior member is reversed (a left) in one case[82] (fig. 126) and in the other[83] it is itself double, the anterior portion being reversed and the posterior homopleural (fig. 125).

The operated limb in all of these cases was composed of two homogeneous halves.

Histories of typical cases are given in the appendix (p. 132).

20. Heteropleural transplantations, dorsodorsal orientation. This combination, being disharmonic, yielded out of seventeen cases twelve duplicities (figs. 127 and 129) and three limbs that became normal by reduction of the reduplicating member (fig. 130). In two individuals[84] normal limbs resulted without external evidence of incipient doubling, and two of the reduplications, in one of which both members are of the same side in linear series, are of an anomalous nature. This makes four cases out of seventeen that do not follow the rule. Two of the combinations are heterogeneous; all of these conform to the rule except one of the anomalous reduplications. The other three non-conforming cases belong in the homogeneous class, and it is interesting that all of the normal cases in this group resulted from the combination of two like halves.

[79] H. E. 2.
[80] H. E. 18 and 21.
[81] H. E. 4.
[82] H. E. 13.
[83] H. E. 5.
[84] H. R. E. 43 and 44.



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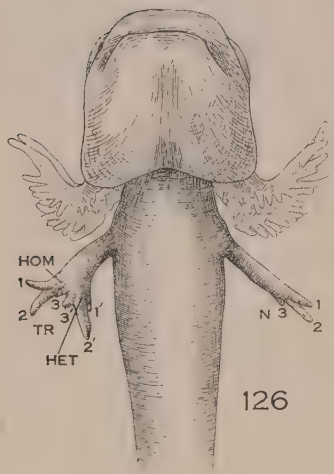

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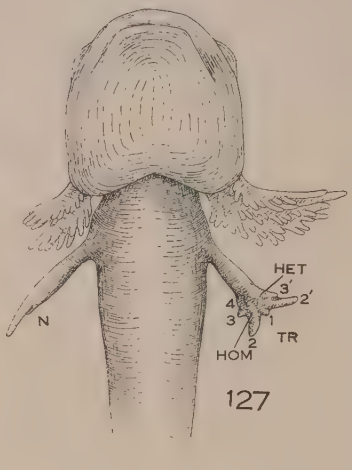

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Fig. 125 Transplantation of half limb bud (comb. 6, fig. 120); posterior half right to anterior right (*hom.dv.*). Exp. H. E. 5. Two limbs, the posterior of which has a double hand. Anterior member an almost normal right; of the two parts of the posterior member, the anterior one is a left hand and the posterior, a right. × 10.

Fig. 126 Same operation (comb. 6, fig. 120) (*hom.dv.*). Exp. H. E. 13. Ulnar reduplication! *HOM*, homopleural hand; *1* to *3*, digits of same; *HET*, heteropleural hand; *1′* to *3′*, digits of same; *N*, normal (unoperated) left limb × 10.

Fig. 127 Transplantation of half limb bud (comb. 10, fig. 120); posterior half right to anterior left (*het.dd.*). Exp. H. R. E. 1. Double hand. *HOM*, homopleural hand with digits (*1* to *4*); *HET*, heteropleural hand with digits (*2′* to *3′*). × 10.

The double limbs are of various degree and kind. The least involved is one in which only the first digit is doubled.[85] In this individual the ventral half of the limb was replaced by a ventral half of the opposite side, and in all probability very little limb material was actually transplanted, since, in the embryo from which the graft was taken, the operated limb developed almost as rapidly as the normal. In five other cases[86] (fig. 127) the whole hand is involved, with indications that in three of these at least[87] the internal reduplication extends farther proximally. In two cases the fore arm and hand[88] (figs. 128 and 129) are externally double, and in one,[89] which was not fully developed when preserved, doubling would probably have shown from near the shoulder down. In two of the individuals[90] there are secondary reduplications. In the two anomalous cases[91] (figs. 131 and 132) there are two entirely separate limbs.

Histories are given on page 134. Unfortunately, external observation does not always reveal the relations of each of the two halves of the bud to the developing members.

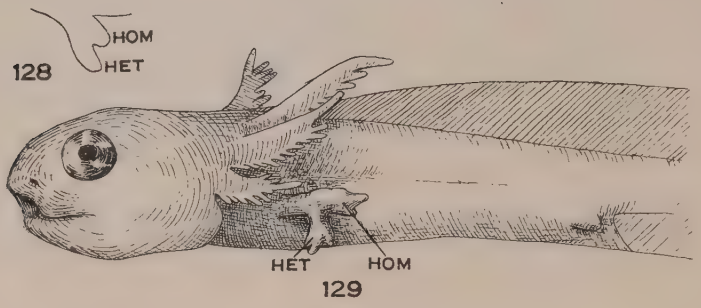
21. Heteropleural transplantations, dorsoventral orientation. Out of twenty-two successful experiments in this group nineteen resulted in the development of normal limbs (fig. 133), which is according to rule, and only three gave rise to reduplications (fig. 134). Two of the latter[92] involved only the radial digits, in which palmar reduplication was present, the limbs being otherwise normal. In the remaining one a bifurcated appendage arose, but the dorsal branch remained merely as a spur attached to the main limb, which was normal though undersized and with slight syndactyly.

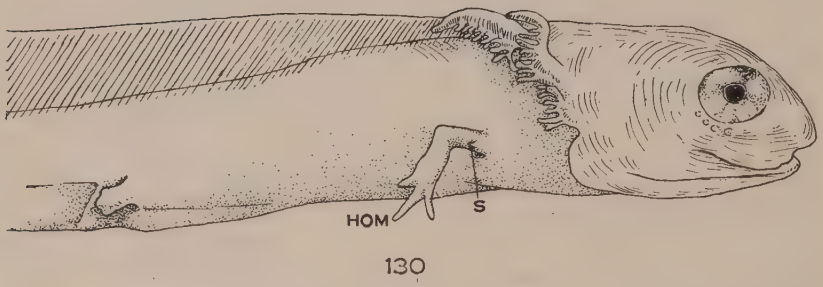
In one case,[93] which has been classed as normal, a filamentous appendage probably not a limb, developed a short distance ventral to the main limb, which was normal though slightly shorter.

[85] H. R. E. 30.
[86] H. R. E. 1, 15, 46, 47, and 48.
[87] H. R. E. 15, 46, and 47.
[88] H. R. E. 5 and 21.
[89] H R. E. 28
[90] H. R. E. 21 and 47.
[91] H. R. E. 9 and 20.
[92] H. R. E. 10 and 16.
[93] H. R. E. 12.

This group of experiments is interesting because two of the combinations (ventral half in place of dorsal and dorsal in place of ventral) are homogeneous. Out of thirteen such cases, normal limbs developed in eleven.

For histories of representative cases see appendix (p. 136).





Figs. 128 and 129 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half right to dorsal left (*het.dd.*). Exp. H. R. E. 5. *HOM*, homopleural member; *HET*, heteropleural member.

Fig. 128 Outline of limb from above, ten days after operation (free-hand sketch).

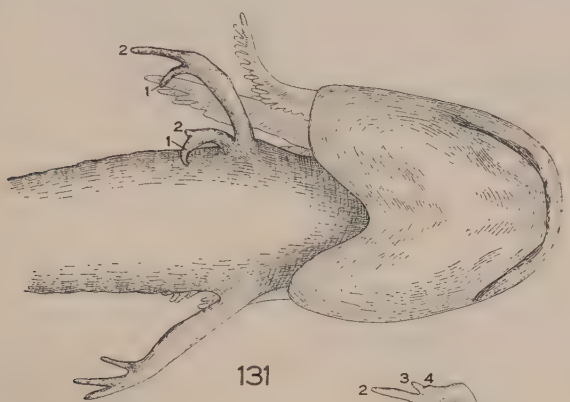
Fig. 129 Preserved specimen lateral view, nineteen days old. × 10.

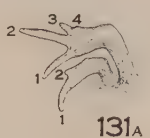
Fig. 130 Transplantation of half limb bud (comb. 9, fig. 120); anterior half left limb bud to posterior right (*het.dd.*). Exp. H. R. E. 11. Normal limb with small spur (*S*). × 10.

22. Discussion of experiments with half buds. In order to establish the conclusion stated in the introduction to this section, that it is the harmony or disharmony of the half-and-half combi-

nation and not one of the particular qualities of the operation that determines whether normal or reduplicated limbs arise, it will be necessary to examine the numerical results of the experiments more carefully.

If we take the actual figures of the experiments and examine the qualities in pairs, we find the actual number of each class and





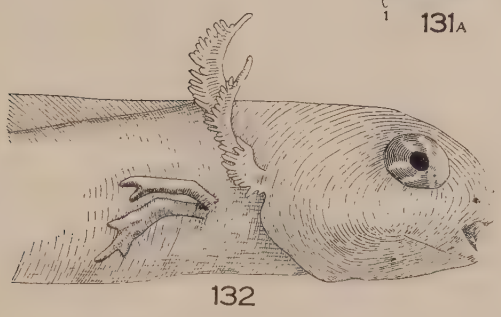
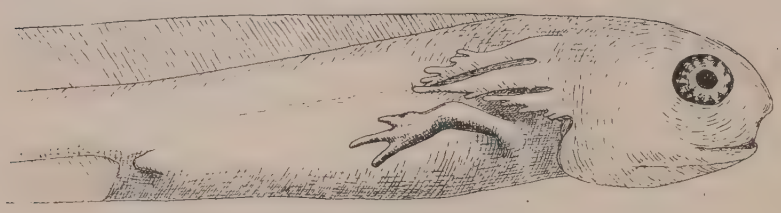


Fig. 131 Transplantation of half limb bud (comb. 10, fig. 120); posterior half left side to anterior right (*het.dd.*). Exp. H. R. E. 9. Anterior member a right (homopleural), the posterior one a left (heteropleural), but imperfect. Ventral view of specimen preserved forty days after operation. × 10.

Fig. 131A Lateral view of limbs of same.

Fig. 132 Transplantation of half limb bud (comb. 11, fig. 120); dorsal half left side to dorsal right (*het.dd.*). Exp. H. R. E. 20. Two right limbs, the anterior one imperfect. × 10.

the proportion of normal results to be as given in table 5, column 6. 'Normal by resorption,' being fundamentally the same as reduplication, is classed as such.



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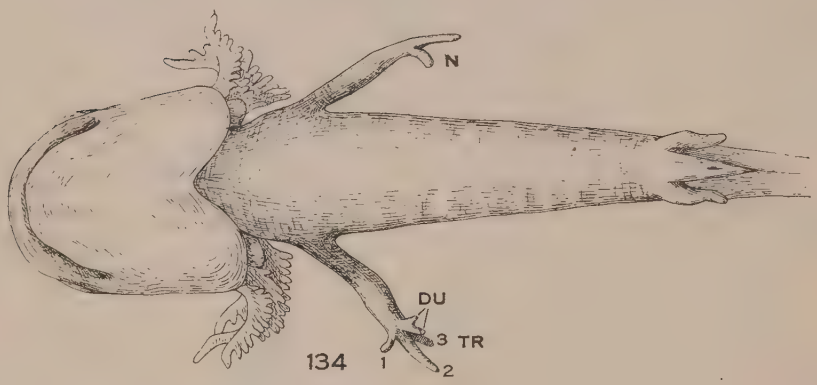

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Fig. 133 Transplantations of half limb bud (comb. 16, fig. 120); ventral half left side to dorsal right (*het.dv.*). Exp. H. R. E. 36. Normal limb.

Fig. 134 Transplantation of half limb bud (comb. 13, fig. 120); anterior half left side to anterior right (*het.dv.*). Exp. H. R. E. 10. *N*, normal left arm; *TR*, grafted arm with palmar reduplication of two digits (*DU*). × 10.

The one thing that stands out is the great difference between the results of the harmonic and those of the disharmonic combinations. In the case of none of the other attributes of operation is there anything like the same difference between those of a pair, though in the case of homogeneity vs. heterogeneity the difference is considerable (37.1 vs. 64 per cent).

However, the comparisons cannot be accurately made without the same number of experiments in each class, unless made by means of percentages. This is quite obvious, for instance, in the case of the homopleural transplantations with dorsodorsal orientation, which all result in normal limbs. The relatively

TABLE 5

Transplantation of half buds. Comparison of pairs of qualities of operation

PAIRS OF QUALITIES COMPARED	NUMBER OF CASES				PER CENT NORMAL	PER CENT NORMAL AFTER CORRECTION FOR INEQUALITY IN NUMBER OF EXPERIMENTS
	Normal	Normal by resorption	Reduplicated[1]	Total		
Homopleural	8	4	11	23	34.8	50.0
Heteropleural	21	3	13	37	56.8	50.2
Dorsodorsal	10	3	10	23	43.5	56.25
Dorsoventral	19	4	14	37	51.4	43.9
Homogeneous	13	7	15	35	37.1	27.7
Heterogeneous	16	0	9	25	64.0	72.5
Vertical	14	5	8	27	51.9	53.75
Horizontal	15	2	16	33	45.5	46.8
Anterior or dorsal half transplanted	15	6	11	32	45.45	50.2
Posterior or ventral half transplanted	14	1	13	28	50.0	50.0
Harmonic (hom. dd and het. dv)	27	0	3	30	90.0	93.9
Disharmonic (hom. dv and het. dd)	2	7	21	30	6.7	6.25

[1] The four cases of anomalous reduplication have been omitted from this tabulation.

small number of cases in this group affects the record for homopleural transplantations by reducing considerably the number of cases that would have developed normally, thereby giving undue weight to the larger number of dorsoventral cases which result in reduplications. Likewise the dorsodorsal vs. dorsoventral record is influenced by the relatively small number of homopleural cases.

On this account the operations in each class have been reduced to a common basis. While the probable error of these figures is in most cases large, the comparisons resulting therefrom are no doubt much more reliable than those resulting from the figures of the actual experiments. They are given in the last column of table 5.

In examining this table we find that there is little or no association between the experimental results and the following qualities of operation: homopleural vs. heteropleural, dorsodorsal vs. dorsoventral, vertical vs. horizontal, anterior and dorsal vs. posterior and ventral, the deviation from total lack of association (50 per cent) being in the most extreme case but 6.1 per cent. When we examine the figures with reference to the pair, homogeneity vs. heterogeneity, we find that there is a much wider difference (27.7 per cent as compared with 72.5). This would have to be regarded as a significant difference but, as will be seen below it is only secondarily so. The marked association between the harmonic combinations and normal development (93.9 per cent) and the very small proportion of normal development (6.25 per cent) in the disharmonic group, show that it is largely this pair of attributes that determines whether development will be normal or not. This quality of harmony or disharmony, however, is not like the simple qualities of side of origin, orientation, or direction of the incision, but is itself a combination of two of them. Those that are harmonic are the homopleural dorsodorsal and the heteropleural dorsoventral combinations, the other two being disharmonic, as in the experiments with whole limb buds.

When we consider the homogeneous and heterogeneous combinations, we find them unevenly distributed with respect to the harmonic and disharmonic. This is on account of the restriction of operation due to the semicircular shape of the transplanted pieces, which makes half of the combinations impossible of execution. Were these all possible, there would be complete symmetry in the aggregation as a whole. In reality, it will be recalled, six of the homogeneous combinations are disharmonic, while only two are harmonic. On the hypothesis that it is the harmony of the combination that determines normal develop-

ment, and with an equal number of experiments in all of the sixteen possible classes, the expectation would be that only 25 per cent of the homogeneous and 75 per cent of the heterogeneous would be normal. This corresponds closely to the figures 27.7 and 72.9, respectively, found in table 5.

As regards the question of equipotentiality, the results of these experiments are equally striking. The two homogeneous combinations which, according to expectation, should yield normal limbs did so. Thus two ventral halves yielded normal limbs in all six experiments, as did two dorsal halves in five out of seven. In three cases of disharmonic homogeneous combination normal limbs developed by resorption of the reduplicating bud; two of these were from two anterior halves and one from two posterior. Two further cases of normal limbs from two anterior halves developed without external evidence of resorption. While the last five, if interpreted according to the rules, can only be accepted as evidence of equipotentiality in so far as they show that a whole limb can develop out of a single half bud, the others show that two half buds which are alike except that they are from opposite sides of the body may give rise, when harmonic, to a single normal limb.

GENERAL DISCUSSION

In this section the following questions will be considered: 1) the foundation of the rules of symmetry; 2) the mode of representation of symmetric relations in the limb rudiment; 3) the formation of reduplications; and 4) form regulation and function in transplanted limbs.

E. The rules of symmetry.

The validity of the rules of symmetry which have already been stated in the introduction (p. 4) will best be realized by considering the results of the several experiments in tabular form (table 6). Conformity is shown most strikingly in the heterotopic group, where there is only a single apparent exception in forty-five cases; and this exception, as already pointed out, is probably due to an error in recording the operation.

In the orthotopic group the lowest percentage of conformity (65.8) is found in the inverted homopleural buds (*hom.dv*), where the exceptions are due entirely to adjustment by rotation of the limb as a whole. In the superposed buds the sole exception is due probably to the same cause. The exceptional

TABLE 6

Showing conformity to rules in the several experiments

OPERATION				RESULTING LIMB				
				Conforming to rules		Exceptions		Total
Type of experiment	Side of origin of graft	Orientation	Harmonic or disharmonic	Number	Per cent	Number	Per cent	
Whole bud heterotopic..	hom.	dd	harm.	7	100.0	0		7
Whole bud heterotopic..	hom.	dv	disharm.	12	100.0	0		12
Whole bud heterotopic..	het.	dd	disharm.	10	100.0	0		10
Whole bud heterotopic..	het.	dv	harm.	15	93.8	1(?)	6.3(?)	16
Whole bud orthotopic...	hom.	dd	harm.	9	100.0	0		9
Whole bud orthotopic...	hom.	dv	disharm.	25	65.8	13	34.2	38
Whole bud orthotopic...	het.	dd	disharm.	31	100.0	0		31
Whole bud orthotopic...	het.	dv	harm.	16	100.0	0		16
Superposed buds.....	hom.	dd	harm.	5	100.0	0		5
Superposed buds.....	hom.	dv	disharm.	4	80.0	1	20.0	5
Superposed buds.....	het.	dd	disharm.	5	100.0	0		5
Superposed buds.....	het.	dv	harm.	5	100.0	0		5
Half buds.....	hom.	dd	harm.	8	100.0	0		8
Half buds.....	hom.	dv	disharm.	15	100.0	0[1]		15
Half buds.....	het.	dd	disharm.	13	86.7	2[1]	13.3	15
Half buds.....	het.	dv	harm.	19	86.4	3	13.6	22
Total.....				199	90.9	20	9.1	219
Average of percentages.....					94.5		5.5	

[1] Four cases of anomalous reduplication have been omitted from this tabulation, inasmuch as they cannot be classified either as conforming or as exceptional.

cases arising after transplantation of half buds have already been discussed, and, as pointed out above, there are in this group of experiments obvious disturbing factors which might readily account for the exceptions. Taking the experiments as a whole, 90.9 per cent conform and 9.1 per cent are exceptional, but if allowance is made for the difference in the number of experiments in each class, assuming that each is a fair sample of what would

occur in a large number of cases, then but 5.5 per cent are exceptional.

The behavior of transplanted limb buds in accordance with the above rules indicates that the posture and the asymmetry of the limb is determined neither by the limb itself nor by its surroundings exclusively, but by an interaction between the two. This is best described by the assumption, that in the stages experimented upon the anteroposterior axial differentiation is already determined within the limb bud, while the ventrodorsal axis (probably radio-ulnar of the grown limb) is determined by its orientation with reference to the surrounding tissues of the host (fig. 135). In a given place a right limb bud upside down thus behaves like a left limb bud right side up and vice versa (fig. 2). It is scarcely necessary to point out that this is not a gravity effect, for the embryo lies on its side during the period when the dorsoventral axis of the limb is determined, 'upside down' being used here merely with reference to the cardinal points of the embryo itself.

What the nature of the influence exerted by the organic environment may be, has not been determined. Whether it acts upon the intimate structure of the limb bud or directly upon the differentiating systems contained therein, without affecting the intimate structure as a whole, cannot be answered from the present data (p. 101). The influence is not sharply localized, for it is the same both in the limb region itself and elsewhere along the flank of the embryo, so that it is probably an effect of the axial differentiation of the tissue elements themselves. It is possible that light may be thrown upon this question by transplanting the limb bud to the dorsal or to the ventral midline of the embryo.

F. The mode of representation of symmetric relations in the limb rudiment

The question whether the adult parts are localized in the germ, forming a mosaic, must be answered in the negative for the limb bud, as used in the experiments, i.e., if we consider as such a disc of tissue, three and a half somites in diameter, centering ventral to the fourth myotome, and leave out of account the outlying regions from which certain portions of the shoulder-

girdle develop. This conclusion is based upon the following evidence derived from the experiments: 1) After extirpation of any half of the limb bud, a complete normal limb may develop from the remaining half; 2) fusion of two limb buds by superposition is followed, if the combination is harmonic, by the development of a single normal limb, which at first is usually larger than normal, but in which there is rapid regulation of size; 3)

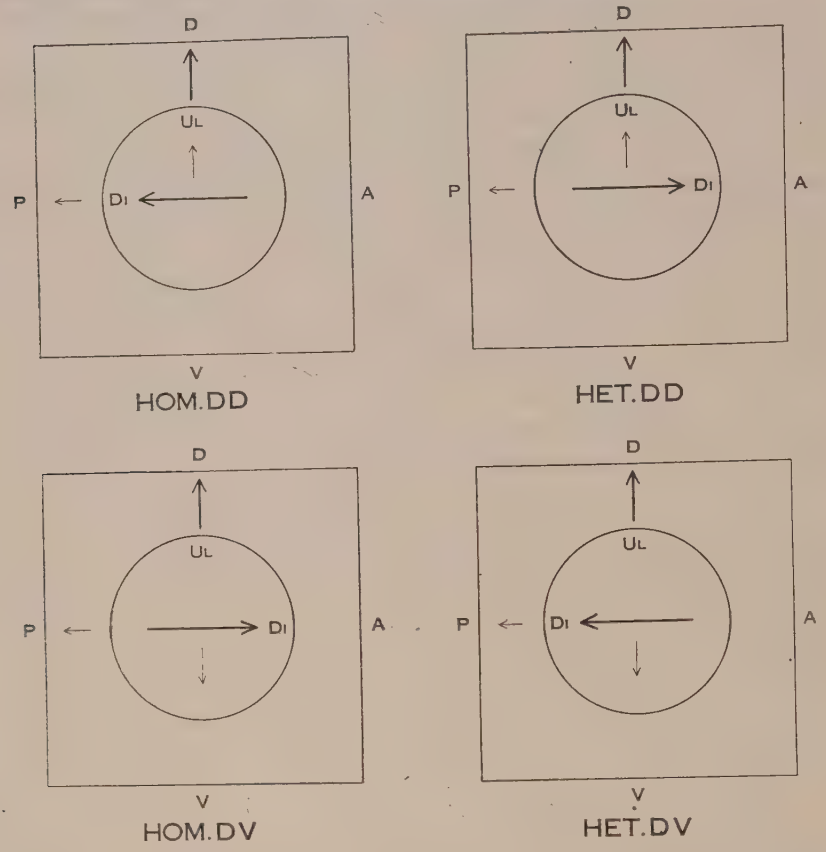

Fig. 135 Diagram to show determination of asymmetry of limb. The circles represent the limb bud, the squares the surrounding part of the embryo. *A*,*D*, *P*,*V*, the cardinal points of the embryo—anterior, dorsal, posterior, and ventral, respectively. The heavy arrows represent the determining axes, i.e., the anteroposterior axis of the bud and the dorsoventral axis of the surrounding parts; *UL*, future ulnar border; *DI*, approximate direction of outgrowth. The smaller arrows show the other axes of bud and surroundings, respectively, which are not effective in determining the axes of the definitive limb.

a normal limb usually develops out of two like halves, i.e., two dorsal, or two ventral halves, if properly oriented, when the opposite half is entirely missing; 4) after inversion of the limb bud the material that normally would have formed the radial half of the limb gives rise to the ulnar half and vice versa, so that practically no part of the bud has the same fate that it would have had if it had been left in place; 5) the inoculation of mesoderm from the limb bud under the skin of the flank of another embryo may result in the formation of a normal limb, although the inoculated tissue is badly disarranged by the operation. According to all tests that have been applied, the embryonic limb rudiment constitutes, therefore, an harmonic equipotential system, though, as a whole, it is self-differentiating except for the determination of its dorsoventral axis. The term 'harmonic equipotential system' is employed here, as defined by Driesch, in the sense that the potencies of all parts of the system are the same, the constituent cells being totipotent.[94] Its use does not imply that the writer attaches to the existence of such systems the same significance as Driesch, who considers them as constituting a proof of the 'autonomy of life.' Even without this, however, and even though the actual system may not reach the abstract perfection demanded by its definition, it remains as a useful conception in experimental morphogenesis. The existence of the equipotential system necessitates, in fact, the assumption of some sort of molecular hypothesis for the representation of adult form in the germ, and herein lies its importance in connection with the present study.[95] In particular, we must look to the constitution of

[94] The concept 'harmonic equipotential system' is defined by Driesch ('05, p. 679) as follows: "Bekanntlich nenne ich harmonisch-äquipotentielle Systeme solche Formganze, bei denen eine Differenzierungs- oder Wachstumsgesamtleistung in ihren Einzelheiten jeweils einzelnen Elementen des Ausgangsganzen zufällt, derart, dass jedes Einzelne dieses Ganzen jedes Einzelne jener Leistung vermag, alles Einzelne aber derart in Harmonie steht, dass die Leistung selbst ein Ganzes ist." The bearing on the question of vitalism is discussed in various papers, especially: '99, p. 99; '01, p. 170; '08 b, p. 138.

[95] Child has expressed skepticism as to the very existence of equipotential systems; for instance: "I think we may say that there is at present no valid evidence for the belief that any living system which is undergoing regulation or development in nature is at any given time an equipotential system" ('11, p. 306). Cf. also Child, '08.

the elementary units of the limb bud, rather than to their arrangement, for the representation of those relations of symmetry that the experiments here described have revealed.[96] In other words, it is the intimate protoplasmic structure that underlies symmetry.

In an equipotential system without axial differentiation, it is most natural to assume that the elements themselves are isotropic.[97] Axial differentiation would then result from the gradual modification of these units by reaction with other elements of the system or through external influences. These differentiations with reference to directions in space may be referred arbitrarily to three axes crossing one another at right angles. They are geometrically of four grades, according to the number of axes along which polarization has taken place.

Taking the models used in stereochemistry to show the spatial relations of the atom groups in certain carbon compounds, we may represent the above four conditions of the elements of the organism or system by four figures (fig. 136) in which the groups that determine the axial relations are situated at the four angles of a tetrahedron. At the center of each tetrahedron we might by analogy assume a carbon atom linked to the four groups occupying the angles of the figure, though this is not necessary for the present purpose. By hypothesis the groups at the angles are supposed to be at first all alike (fig. 136, *1*). If one of them should be changed by some reaction, the structure of the molecule would become polarized (fig. 136, *2*), and if all the molecules should assume approximately the same orientation, the system which they constitute would show a similar polarity. If two of

[96] The question whether relations of symmetry of the organism are to be based upon symmetrical relations of the intimate protoplasmic structure is answered in the affirmative by Driesch ('08 a, p. 144): "Wir müssen also alle Symmetrie und auch alle Wirkungen, die von äusseren Faktoren ausgehen und sich auf Symmetrie beziehen, auf präformierte, gerichtete Elemente des 'Protoplasmas' beziehen und können in jenen Wirkungen nur richtende und umordnende Geschehnisse sehen.

[97] To avoid misunderstanding, it should be stated that when we speak of equipotentiality and isotropy, we do not lose sight of the fact that the system in its entirety is heterogeneous.

the groups become differently modified, then the structure becomes bilaterally symmetrical (fig. 136, *3*). And, finally, if three become modified, so that all four are different, then the arrangement becomes asymmetrical (fig. 136, *4* and *5*) as in the case of optically active substances with an asymmetric carbon atom. In the last phase there are two kinds of individuals, which are exactly alike in every respect, except that they are the mirror images of

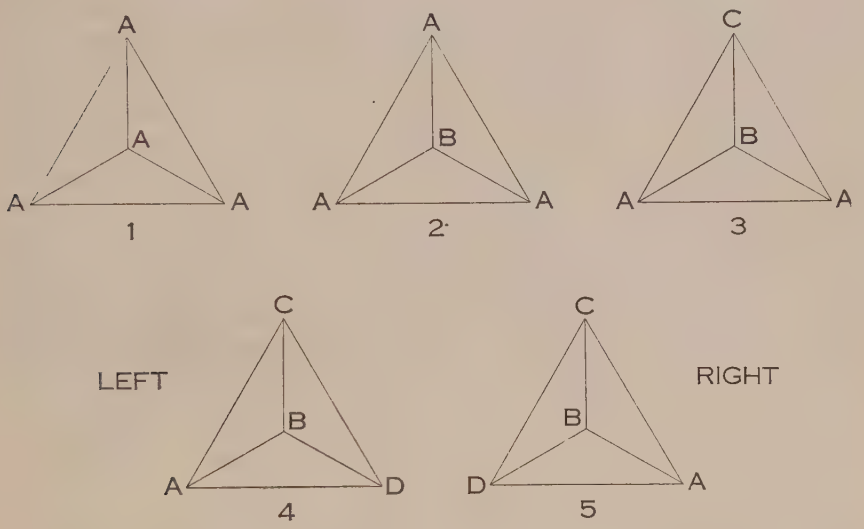

Fig. 136 Diagram to show hypothetical progressive differentiation of the structural units. 1) condition of isotropy; 2) polarization with reference to one axis; 3) bilateral symmetry (two axes differentiated); 4 and 5) condition of complete asymmetry (three axes differentiated) giving right and left enantiomorphs.

one another—in other words, rights and lefts. This is expressed in aggregate form in the right- and left-handed crystals corresponding, respectively, to the dextro- and laevo-rotatory forms of otherwise identical substances.

The experiments with limbs show that the bud at the time of transplantation is in either the second or the third phase, probably the former. There must be a differentiation along the antero-posterior axis, because if this is reversed the limb shows it by growing in a direction nearly opposite the normal. The medio-

lateral axis is probably not differentiated, though in the absence of sufficient experiments in reversal of this axis, it is better to make no definite assumption regarding the intimate structure in relation to it. The dorsoventral axis is at most but slightly differentiated, and if it is at all, then the differentiation is reversible.[98] As already pointed out (p. 55), there is some ground for the latter assumption, for it has been observed that after transplantations in the heteropleural dorsoventral position (the harmonic combination in which the dorsoventral but not the anteroposterior axis is reversed) the adjustment of the tissues of the limb bud is apparently not immediate, but involves a time factor, probably not entirely accounted for by the effect of the operation as such. Whatever the character of this dorsoventral differentiation may be, it is nevertheless very slight in comparison with the anteroposterior differentiation, which has become irreversible by the time the stage in question is reached.

If we could experiment over a wide enough range of stages, it should be possible to determine the time limits of the above phases of axial differentiation of the limb rudiment. At present, however, there are no data bearing upon the question, for in the earliest stages in which the transplantation of limbs has been carried out (embryo with wide open medullary folds), as shown by Detwiler ('18), the limb bud follows in its development the same rules as here formulated.

Lest the foregoing scheme seem too formal, it may be pointed out that the model has been chosen to explain solely the relatively simple characters of polarity and symmetry. Upon this as a basis, further experimentation may yield facts from which the mode of representation of more specific form features may be determined. There is nothing in such a scheme inconsistent with the fact that the cell itself is not a homogeneous system, for the model is supposed to represent only that constituent of the system which determines the adult character in question.

[98] This is perhaps odd in view of the facts brought out by Przibram ('10 b), showing that dorsoventral differentiation is very marked in the animal organization, more so, for instance, than the anteroposterior differentiation.

The point which it is desired to emphasize is that in an organic 'equipotential system' there must be some intimate structural basis for adult characters in the units that make up the embryonic rudiment.[99] It cannot be in the arrangement of these units; for in that case marked disturbances of development would be produced by such operations as removing half of the rudiment, fusing two buds together, combining two like halves, inverting the dorsoventral axis, or inoculating masses of mesoderm cells from the limb rudiment under the skin of the flank; and yet normal development may follow any of these procedures.

These experiments yield, of course, no information concerning the localization in the cell of the representatives of the adult form characters in question. The system here dealt with is a pluricellular one, but it is interesting to find that in the most thorough and careful studies of polarity and symmetry in the egg, the basis of these properties is found to be in the cytoplasm of the egg cell. Lillie ('06, '09) shows that the polarity of the Chaetopterus egg must be located in the ground-substance, because any amount of shifting of visible granulations in the egg, such as yolk, oil droplets, pigment, etc., has no effect on the polarity of the resulting embryo. With this conclusion the work of Morgan and his collaborators ('08 b, '09, '10) on the centrifuged eggs of various animals, more particularly Arbacia and Cumingia, is in substantial accord. Conklin ('16, '17), in his study of Crepidula, concludes that it is the spongioplasmic framework of the egg-cell that determines its polarity, though he does not consider how this quality is determined in relation to the intimate structure of the spongioplasm. To the extent that Conklin places the seat of polarity in the more viscid rather than in the more fluid constituent of the cytoplasm, he takes issue with Lillie, but in the main, there is agreement between these two investigators. Lillie, however, goes a step further when he says ('09, p. 77): "The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when

[99] Cf. Driesch, l. c.

the yolk granules are packed closely into the small cell of the two-celled stage of Chaetopterus) seem to me to argue for a molecular basis of the fundamental principle of vital organization." Morgan, likewise, takes this view when he says ('09, p. 114), "These considerations incline us to the view that there exist in the molecular constitution of the egg the potential factors of symmetry." The scheme outlined above is in harmony with this concept.

On the other hand, Child ('13, '15), as also Della Valle ('13) rejects all such hypotheses, basing the phenomena of axial differentiation upon the occurrence of gradients, which, according to Child, are primarily of a functional (metabolic) nature. It seems to the present writer that such gradients may well be an expression of the polarity rather than its cause.

G. Reduplication and the problem of polarity and heteromorphosis

The reduplications which have been observed in the various experiments have already been described sufficiently for the present discussion (pp. 35, 45, 65, 73). The salient facts are: 1) that the duplicate is the mirror image of the original limb; 2) that more than one secondary member may arise by budding from the same primary bud, in which case both of the former stand in some relation of symmetry to the original; 3) that the secondary appendages themselves may be doubled, forming a more or less symmetrical pair. There are a few exceptional cases, where two members of the same side stand in linear series, but probably these have arisen only where the two rudiments are far enough apart not to influence one another.[100]

[100] Several cases are to be considered here. One (H. R. E. 10-) is a case in which the anterior half of the limb bud was removed. Two limbs developed, one clearly from the remaining posterior half and the other probably from the anterior border of the wound (cf. Harrison '18, p. 441). The operation was done on the left side and both limbs were lefts, the posterior one being somewhat defective. In another case, which had an early history similar to the above, the posterior member was very defective and it was impossible to determine whether it was a right or a left. A third case (H. R. E. 20) is the one figured on page 79. This is not a case of regeneration, but one in which the anterior member probably developed from the grafted half, while the posterior member may have developed from the stationary ventral half. Both are attached to the same shoulder-girdle, but there are two separate glenoid cavities.

Bateson, in his "Materials for the Study of Variation" ('94), has given an exhaustive review of the literature relating to supernumerary parts, in which the limbs are fully considered. In this treatise he has made a masterly analysis of the available material, particularly with reference to the appendages of arthropods. The phase of the problem which is especially relevant to the present discussion is that concerning what Bateson calls minor symmetries, in which the supernumeraries are in some way symmetrical with respect to themselves or to the normal appendages with which they are associated. The other class of supernumeraries, in which two identical appendages stand in simple succession to one another are, according to Bateson, practically unknown, and even those that have been described are considered by him to be of somewhat doubtful nature, though many cases of simple hyperdactyly would seem to belong in this category.

The symmetrical extra appendages fall into two groups: 1) those in which there is a pair of extra members symmetrical with themselves, arising from the normal appendage with which one of the supernumeraries appears to have a definite relation of symmetry, and, 2) those in which the single supernumerary is symmetrical with respect to the normal. The former condition Bateson considers to be the more usual, and, in fact, he accepts the existence of the latter with a certain skepticism which seems unnecessary.[101] It is true that many cases that apparently fall within the latter group may upon closer examination be found to belong in the former, but the converse is also true, as will be shown below (p. 97). Bateson has devoted special attention to the first group, and, on the basis of about one hundred and twenty cases in insects and a considerable number in the Crustacea, he has formulated the following rules,[102] showing the relation of the supernumerary appendages to each other and to the original member:

I. The long axes of the normal appendage and of the two extra appendages are in one plane: of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other is remoter from it.

[101] Op. cit. p. 539 and 553. Consider, however, in this connection, the clear case described by Bender ('06).

[102] Op. cit. p. 479.

II. The nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

Transverse sections of the three appendages taken at homologous points are thus images of each other in parallel mirrors.

In the vertebrates Bateson marshals a large amount of material, of which about fifty cases are in amphibians.[103] At the time Bateson's book was written, however, little or nothing was known regarding the origin of supernumera y appendages in either the arthropods or the vertebrates. Since then a large amount of experimental evidence has accumulated to show that they may be formed by superregeneration, especially by regeneration from complex or irregular wound surfaces.[104] The evidence all corroborates Bateson's main gene. alization regarding the relation of symmetry of supernumerary limbs, and there are practically no exceptions.[105]

The importance of double supernumeraries (Bruchdreifachbildung, la doppia rigenerazione inversa, see p. 95) is emphasized in the papers by Emmel ('07) and Della Valle ('13), and this conception is given prominence in Przibram's more general discussion of the question ('09, p. 234).

[103] See Bateson (pp. 554–5) for a discussion of the older literature.

[104] In the amphibians the investigations of Barfurth ('94), Giard ('95), Tornier ('97, '00, '05, '06), Lissitzky ('10), Fritsch ('11), Kurz ('12), Della Valle ('13), and others have added much to our knowledge of the subject. In the crustaceans Przibram ('02), Reed ('04), Zeleny ('05), and Emmel ('07) have reported experiments which, though not so numerous, are none the less important. The more recent literature is fully discussed in many of these papers, especially in those of Lissitzky, Fritsch, and Della Valle, to which the reader is referred for details.

[105] A remarkable exceptional case has recently been described by Dawson ('20) in a lobster, in which there is an extra pair of chelipeds attached to the normal. The two extra chelae are mirror images of one another, but the one nearer the primary claw is not mirrored from the latter, but is of the same side. Furthermore, the primary claw is a 'nipper,' while the supernumeraries are both of the 'crusher' type, so that the case proves to be likewise an exception to Przibram's rule ('11), according to which, in heterochelous forms, the extra appendages are of the same type as the primary. The case described by Cole ('10), also in a lobster, is an almost diagrammatic example of Bateson's rule, if allowance is made for the effects of torsion.

Likewise, the well-known experiments of Tornier ('05) upon tadpoles of Pelobates, in which the hind-limb bud was divided in an early stage, some of the cases of Lissitzky ('10), and Della Valle's case of reversed regeneration conform closely to Bateson's rules. Although the end results of the experiments of Tornier and of Della Valle are analogous, there is, however a sharp difference of opinion regarding the exact mode of origin of Tornier's double supernumerary hind legs, Tornier maintaining that they both arise from the dorsal part of the pelvis, which was split off by the operation, while Della Valle holds them to be analogous to his own case.

Della Valle has laid particular stress upon the supposed identity of change of asymmetry and reversal of polarity, and has sought to make the various cases of superregeneration which have been reported fit into his scheme of 'doppia rigenerazione inversa.' The case on which Della Valle bases his discussion of these questions is that of a newt (Triton) in which the left anterior limb was fractured in the region of the brachium and cicatrization prevented by tying a silk thread around the limb at that level. Twenty days later the same limb was amputated a short distance below the point of fracture. There regenerated three perfect limbs, one from the distal end of the stump and two from the region of the fracture. Of the latter, one was from the proximal end of the small portion beyond the ligature and the other was apparently a continuation of the stump proximal to the fracture. The first and last of the three were left limbs, i.e., of the same side as the original, while the one which regenerated in a distoproximal direction was reversed. The end result was a triple appendage in which the three members were placed in accordance with Bateson's rule.

Della Valle seeks to make the cases of Tornier ('05) and Lissitzky ('10) conform to this scheme, and falls into line with Przibram ('09) who had previously given a schematic representation of the same phenomena, which he termed 'Bruchdreifachbilding.' He also interprets in the same way the reduplications obtained by Braus ('04, '09) and myself ('07) in the transplantation of embryonic limb buds. He suggests that when the limb

bud is implanted in normal location, triplicate appendages could be accounted for in the following way: one member derived directly from the grafted bud; one member, of mirror symmetry, by an inverse regeneration from the base of the bud, and the third member, of original asymmetry, from the wound surface of the host. This view is, however, not borne out by the present experiments.[106]

When the whole of the evidence bearing on the question is taken into consideration, one cannot but think that too much weight has been placed by Bateson and his followers on the double supernumerary. The other class of cases, where the single supernumerary is symmetrical with the normal appendage with which it is associated, while neither so numerous nor so spectacular, is nevertheless of wide occurrence. Cases reported by Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), and Megušar ('07) show that truly double appendages in mirror symmetry with respect to each other may be formed by constric-

[106] Se dunque noi considerassimo uno di questi innesti praticati invece che in una regione lontana (come p. es. nell'orbita), nell'immediata vicinanza della regione donde fu tolto l'innesto, noi osserveremmo l'uno presso dell'altro lo sviluppo oltre che dell'arto normale, anche dell'arto rigenerato dalla superficie di sezione della regione prossimale del corpo, nonchè dell'arto sviluppatosi dalla superficie di sezione della regione periferica, identico all'arto che lo ha prodotto, ma con simmetria speculare. La identità anche di questo fenomeno con la doppia rigenerazione inversa dalle due superficie di una ferita risulta in questo modo evidente. Della Valle: op. cit. p. 125.

There is opportunity to test this hypothesis by comparing the experiments in which the wound-bed was cleaned with those where it was not. In the former, regeneration from the host is precluded (p. 6), and triplicate limbs could only arise by a second reduplication from the base of the graft; whereas in the latter, regeneration from the host should occur in a large number of cases, if at all, and thus yield a large proportion of triplicate appendages. An examination of the results shows that this is not the case. In the first place, as shown in table 2, the total number of reduplications in the series with cleaned wounds is fifty-three, which is 56 per cent of the total number of positive experiments, while there are but sixteen cases (33.3 per cent) in the group with non-cleaned wounds. The disproportion is much greater when the number of triplicate appendages in each group is compared. Out of a total of eighty-seven cases old enough to be determined, there are twenty-five triplicate limbs (28.7 per cent) in the clean-wound experiments and only three in forty-eight cases (6.25 per cent) in the others. It is quite clear, then, that leaving in the wound-bed cells that are capable of giving rise to a new limb reduces greatly, instead of increasing, the chance of formation of supernumerary limbs, so that Della Valle's suggestion is untenable.

tion of a simple regenerating bud. This harmonizes with Driesch's ('06) observations on double Echinus embryos.

In the present work, the reduplicated extremities are nearly all found to be in minor symmetry, and many of those in which three members are present, if seen only in the fully developed condition, would appear to be cases of paired supernumeraries, conforming, though with some aberration, to Bateson's rules. The individual histories show, however, that they are mostly simple duplicities in which the supernumerary mirrors the original, and this seems to be the case in Braus's experiments, too. Two reduplicating limbs often do develop, but usually each grows as a bud from the original instead of the two arising as a pair in themselves. Each of them mirrors the original limb, so that the two supernumeraries are both of the same side. In other cases the supernumeraries are themselves double, in which event there is strict conformity to Bateson's rule, but the former constitute a large majority, and conformity there is only superficial, for the original limb is the middle member and not one of the extremes.

In view of these facts, there is probably no very fundamental difference between the two classes of reduplications, i.e., between the double supernumeraries symmetrical with each other and the single supernumerary symmetrical with the original; had Bateson had the developmental stages at his disposal, he himself might not have drawn so sharp a distinction.

In accordance with the above, Bateson's rules might be stated in more general form, so as to include both simple duplicities and symmetrical pairs, as follows:

1. The long axes of duplex or multiplex appendages lie in one plane.

2. Two adjacent members form in structure and position the image of each other, as reflected from a plane mirror bisecting the angle between the respective axes and perpendicular to the common plane of the two axes (figs. 3 and 4).

The present experiments show (tables 2, 3, 5, and 8) that, excepting heterotopic grafts, it is in the disharmonic combinations that reduplications are most frequent. What, now, is the nature of the disturbance that causes the doubling of transplanted

limb buds and of regenerating limbs, which, when it occurs, is always combined with reversal of one member? The first visible sign of reduplication both in the embryonic limbs and in the regenerating blastema is the presence of two growth centers for the limb in place of one; each becomes an apex of growth, with a resulting bifurcation of the appendage as a whole. The question arises whether the doubling of the growth center is antecedent to or resultant from the reversal of the asymmetry. From the fact that mere mechanical division of a simple regenerating center[107] may bring about doubling, it would seem to be more probable, if not certain, that the existence of two growth centers within spheres of mutual influence is the factor that produces the reversal in one—the one that is less advantageously placed, or in which differentiation is less advanced.

The problem before us thus resolves itself into two phases: that of division or repetition of parts and that of symmetry. This was clearly seen by Bateson, who has emphasized the fundamental nature of the power to divide.[108] No attempt will be made here to analyze this phase of the question. The symmetric relations of the repeated parts are, however, so definite and of such general recurrence that they, too, are beyond question of a fundamental nature.

The phenomenon of reversal of asymmetry has been treated by many investigators as one with that of axial heteromorphosis, and yet this is not strictly correct, for the reversal of asymmetry may be brought about by the interchange of the poles of any one of the three axes to which the object is referred, and not necessarily the one along which regeneration and differentiation is taking place. This is true not only when regeneration occurs in a proximodistal direction, as in the cases of Tornier, Zeleny, and others, cited above, but also when it takes place distoproximally, as shown in the two experiments reported by Kurz ('12).[109]

[107] Cf. Tornier ('97), Przibram ('02), Reed ('04), Zeleny ('05), Megušar ('07).

[108] "This power to divide is a fundamental attribute of life and of that power cell division is a special example." (Problems of Genetics, p. 38.)

[109] In somewhat similar experiments by Morgan ('08 a) only the bone, not the soft parts, was reversed. Nothing is said regarding the exact character of the limbs regenerated.

The fundamental phenomenon, therefore, is not that a particular axis is reversed, but that reversal occurs at all, and how it is brought about.

Organic polarity, in general, has been based either on the supposed polarization of the organic units themselves or upon a supposed gradient of a functional (Child) or material (Morgan, '05) nature, running from one end of the organism to the other. There is evidence for the occurrence of both factors, and what seems most likely is that both are at play. Under certain circumstances they act in the same direction; under different conditions one may antagonize and retard, or even overcome, the other, as seems likely in heteromorphic regeneration where polarity is reversed (earthworm, planarians, amphibian tail, etc.). Przibram ('13), who advocates a theory combining the two factors, which he calls 'Richtungspolarität' and 'Schichtungspolarität,' respectively, nevertheless regards the reversal of polarity as due to actual rotation of the cells. He ('06, '10 a) cites unpublished work by Hadži in support of this view, and adopts it in his diagrams ('09) illustrating the five fundamental varieties of operation leading to regeneration (Biotechnik).

The figures are not convincing, however, for just as much rotation of the cells is shown at the end where polarity is not reversed as at the other end where it is reversed (Przibram, '09, pl. XV, *1h–3h*), and in fact, as expressed in these diagrams, what turning is shown is nothing more than a wound-healing process. Until it is demonstrated that rotation of the cells as a whole takes place solely in heteromorphic regeneration, it cannot be used to explain reversal of polarity.

So long as the elementary units of the limb bud have one plane of symmetry left, and the final asymmetry of the limb remains to be determined by its relation to certain axes of the embryo,[110] it

[110] In the case of asymmetric organisms, the elementary units, if representing the form of the organism at all, must be postulated as asymmetric themselves. In the case of paired organs, each asymmetric in itself, but symmetrical with respect to its opposite, polarization on the transverse axis may be assumed as due to the position of the parts with respect to the other two axes (cf. Przibram, '13, p. 38) and not as necessarily due to actual differentiation of the elements in the transverse direction.

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will of course be possible to account for its reversal by rotation of the elements about the proper axis. As an alternative to the rotation hypothesis, we might, however, consider reversal as due to an interchange in position of two of the determining groups in the elementary units (p. 89, fig. 136). In case of differentiation on all three of the axes, i.e., if the units themselves are asymmetric, then reversal could take place only in the latter way, unless it occurs altogether independently of the intimate structure.

There is an analogy for reversal of this kind in the change of the asymmetry of organic molecules of known composition, as, for instance, in the Walden inversion by means of successive substitutions, or in the conversion of dextrotartaric acid into racemic acid, by which transformation half of the dextrorotating groups are changed into the laevo form. Of course, these examples are mere analogies.

Such questions have been touched upon by many of those who have studied twins and double monsters, but, unfortunately, the evidence both as to the cause and as to the occurrence of reversal of asymmetry is conflicting. In the case of human duplicate twins, it is certain that there is no situs inversus viscerum, except very rarely, and apparently even in double monsters the degree of fusion of the two individuals must be considerable for the asymmetry of the internal organs—heart and alimentary canal—to be reversed. On the other hand, it has been shown by Wilder ('04) that in duplicate twins the friction-skin patterns of the two mates may show mirror imaging, particularly those on the index fingers. A similar condition has been found by Newman ('16) in his study of variation of the scutes in armadillo quadruplets, except that here the matter is further complicated by the relation, in pairs, of the four individuals of a litter.[111]

[111] "Now in the armadillo there are many definite evidences of a system of symmetry common to all of the quadruplets, upon which has been superimposed a secondary symmetry system between twins. This in turn is more or less completely obliterated later by a tertiary symmetry between the antimeric halves of the single individuals. In some sets evident traces of the primary system of symmetry persist as mirror-image relations between individuals of opposite pairs, but it is more usual to find no trace of the primary system. The secondary mirror-imaging between pairs is far more commonly in evidence, but is frequently

The evidence which Morrill ('19) has collected from the study of double monsters in fish embryos shows that situs inversus does occur, but that it is the exception, not the rule, and that there is no "very precise relation between the amount of separation of the two components and the occurrence of mirror imaging."[112] This would seem to oppose the view expressed above, that it is the proximity of two growth centers that causes the reversal of one. Still, in the absence of statistical data regarding the correlation of the two events, it is unsafe to draw a definite conclusion.

In this connection the recent work of Spemann and Falkenburg ('19) is of the greatest importance. By extension and modification of the earlier methods of the former, these investigators obtained a large number of twins in Triton by constricting the eggs in segmentation stages or in the early blastula. They found that in a large number of the cases one individual of a pair (the right-hand member in all cases but one) has complete situs inversus viscerum. Spemann, after an admirable critical analysis of the question, reaches the conclusion, that while some asymmetric intimate structure must be postulated to account for the normal asymmetry of the vertebrate body, there is no proof from these

obliterated by the tertiary mirror-imaging between antimeric halves of the same individual, which latter is the prevailing symmetry system. . . . . In general, mirror-imaging between individuals of opposite pairs is interpreted as an evidence of the early system of symmetry present in the embryonic vesicle before polyembryonic budding began. When the primary buds are formed they are the product of the antimeric halves of the undivided embryo and therefore should have mirror-image relations, but a partial physiological isolation of the two buds permits a certain degree of reorganization or regulation in the symmetry relations, that tends partially to obliterate the original symmetry relations of the undivided embryo. Similarly, when each primary bud subdivides to form the secondary buds that are the primordia of the definitive individuals, a certain residuum of the primary bud symmetry system is carried over, manifesting itself in mirror-imaging between the twins derived from the same primary bud. But here again a certain amount of regulation occurs so that a third system of symmetry, the bilateral symmetry of each individual, tends to obliterate former systems of symmetry." Newmann: op. cit., pp. 200–201.

[112] Tannreuther ('19, p. 359) has recently figured a double chick embryo in which the two individuals are united only by the posterior tip of the primitive streak. Although trunk and head are entirely separate, the heart of the embryo on the left shows situs inversus. No mention is made of this fact in the text.

experiments that it has been completely reversed by the operation. The evidence points, on the contrary, to the introduction of another factor which is manifested also in the tendency of the individuals to show defects on the inner side (i.e., the side turned toward the partner). In the case of the left-hand member, this acts in the same sense as the innate tendency to asymmetry of the viscera, while in the case of the right-hand member, it antagonizes, and in many cases, overcomes the latter.[113] Spemann suggests many different experiments to throw light upon this question. The interesting point in Spemann's discussion, in connection with the present work, is that the necessity for assuming some sort of intimate structure to account for external symmetry relations is recognized. In the case of the reduplicated limbs it is not clear whether the reversal of the secondary bud is a result of direct action upon the individual processes of development going on within it, or whether the influence of the primary bud actually reverses the intimate structure. If it should be found that the reaction takes place before the cells of the limb blastema lose their totipotence, then the latter is undoubtedly true. Otherwise it may be that the differentiation in the limb blastema takes place directly under the influence of the tissues of the environment.

H. Form regulation and function in transplanted limbs

That the limb bud after transplantation becomes adapted in a measure to the new conditions is obvious from a casual consideration of the experimental results. There are different types of adaptation, however, for although regulation of form and function go largely hand in hand, in some cases there may be very complete functional regulation without form regulation,[114] and, particularly in the heterotopic grafts, form regulation without function. By form regulation is meant, in the present connection, the process by which a limb bud that is implanted abnor-

[113] Cf. also Pressler ('11).

[114] For instance, in Experiment I. E. 64, the single disharmonic limb functioned very actively and effectively.

mally in relation to the embryo as a whole becomes adjusted so as to give rise to a limb in harmony with its new surroundings. Perfect adaptation is attained, however, only in cases where there is functional adaptation as well, i.e., only when both form and function are adjusted to the organism as a whole, and this occurs only in the orthotopic position or in positions very close to it. Functional regulation is here largely a question of innervation, and, inasmuch as this has been made a subject of special investigation by Detwiler ('19, '20), it will not be considered at present further than is necessary in its relation to form regulation.

Considering only the orthotopic grafts, there is no a priori ground for expecting any of the combinations to yield normally functional limbs except the homopleural dorsodorsal group, in which the axial relations of the transplanted bud remain normal with respect to the cardinal points of the embryo. Nevertheless, it has been found that inverted limb buds from the opposite side of the body yield almost as high a proportion of normally formed and normally functional limbs as the first-named group. This phenomenon has been interpreted as due not so much to a secondary regulation as to the structure of the elementary units of the limb bud, which are supposed to be symmetrical or reversibly differentiated (p. 89) along their dorsoventral axis. With an equal number of experiments in each of the four groups and without any disturbing factors, the primary processes of regulation should therefore lead, in accordance with the fundamental rules of symmetry, just as often to adaptive (harmonic) as to nonadaptive (disharmonic) end results, but not more often. This proportion is, however, exceeded by nearly 20 per cent of the expectancy, so that, taking the orthotopic experiments as a whole, 59.6 per cent yielded normally functioning limbs in normal posture, while 40.4, for the most part reduplications, were nonadaptive or only imperfectly adaptive (table 7). The proportion is almost the same in the three classes of experiments, whether with whole buds after extirpation of the normal bud, with superposed buds, or with half-buds, though, as more fully discussed below (p. 107), it is quite different in the heterotopic grafts.

TABLE 7

Showing the adaptive or non-adaptive character of the resulting limbs after orthotopic transplantation

TYPE OF EXPERIMENT	TOTAL NUMBER	ADAPTIVE (SINGLE HARMONIC LIMBS)				NON-ADAPTIVE (REDUPLICATION OR SINGLE DISHARMONIC LIMBS)			
		Primarily	By secondary regulation	Total	Per cent	Primarily	By secondary regulation	Total	Per cent
Whole buds									
hom. dd	9	9	0	9	100.0	0	0	0	0.0
hom. dv	38	0	10	10	26.3	25	3	28	73.7
het. dd	31	0	5	5	16.1	26	0	26	83.9
het. dv	16	15	0	15	93.8	0	1	1	6.3
Total	94	24	15	39	41.5	51	4	55	58.5
Average of percentages					59.0				41.0
Superposed buds									
hom. dd	5	5	0	5	100.0	0	0	0	0.0
hom. dv	5	0	1	1	20.0	4	0	4	80.0
het. dd	5	0	1	1	20.0	4	0	4	80.0
het. dv	5	5	0	5	100.0	0	0	0	0.0
Total	20	10	2	12	60.0	8	0	8	40.0
Average of percentages					60.0				40.0
Half-buds									
hom. dd	8	8	0	8	100.0	0	0	0	0.0
hom. dv	17	0	4	4	23.5	13	0	13	76.5
het. dd	17	2	3	5	29.4	12	0	12	70.6
het. dv	22	19	0	19	86.4	0	3	3	13.6
Total	64	29	7	36	56.3		3	28	43.8
Average of percentages					59.8				40.2
Total harmonic	65	61	0	61	93.9	0	4	4	6.1
Total disharmonic	113	2	24	26	23.0	87	0	87	77.0
Grand total	178	63	24	87	48.9	87	4	91	51.1
Average of percentages[1]					59.6				40.4

[1] As given in the three main groups in the upper part of the table.

The secondary regulation of form may be brought about in one of two ways: either by rotation of the limb as a whole during development, whereby it is gradually brought into normal posture, or by a process of reduplication, which is more complicated. In the latter the original grafted limb bud gives rise to a secondary or reduplicating bud of mirror symmetry, which outstrips the former in development, reducing it to a spur or even practically suppressing it. The process of reduplication more often yields, however, actual double appendages, and there is no hard and fast line between the latter and the single limbs with the original bud reduced to a spur. The figures given in the table are therefore somewhat arbitrary in this respect.

Regulation by rotation has been noted only in the inverted limb buds from the same side of the body (*hom.dv*), and it has been further shown that this mode of adjustment probably occurs when the limb bud at the time of operation is rotated anteriorly along the dorsal semicircumference somewhat less than 180° (p. 41). The mechanics of this process is not yet understood. In the other disharmonic group single limbs are very rare (only one case), unless reversal by reduplication and reduction occurs, and no cases of rotation have been observed.

Regulation by reduplication and reduction, which is attended by reversal of asymmetry, has been observed in five cases out of thirty-one in the heteropleural dorsodorsal group, but no perfect cases of single limbs so produced have been found in the other disharmonic combination (*hom.dv.*). In the latter group, however, reduplication is frequent, and the reduplicating member is often normally attached and assumes the same posture as the normal limb, but it has never been found to begin its development early enough to bring about the suppression of the original bud.

In a measure offsetting the regulative cases, there are four others in which the same process, reduplication, has resulted not in regulation, but rather in its prevention. In three cases of inverted buds[115] (*hom.dv.*), where there was tendency to regulate by rotation, this regulative process was rendered futile by secondary budding, and also in one case of a heteropleural dorso-

[115] I. E. 86, 88, 90 and possibly two other cases. See p. 37.

ventral graft, the usual primary regulative result was vitiated by reduplication. The same was true in three cases of half-buds inverted on the opposite side of the body (*het.dv.*). Reduplication, therefore, is not in itself a regulatory process, but leads merely to a condition where regulation may take place through the reduction of the disharmonic member.

The question now arises, whether the experiments give any ground for assuming that there is anything teleological in these regulatory processes. While there is no proof that regulation by rotation is not of this nature, it would be a mistake to draw any conclusion regarding it until the details of the process are better understood. With regard to regulation through reduplication and reduction, it is clear that mechanical factors are sufficient to account for the process. It is in the disharmonic combinations that secondary regulation is necessary to produce normal results. When this is not accomplished by rotation, reduplications almost always arise, and there are only two cases of single disharmonic limbs among the orthotopic grafts. The disharmonic relation, which is brought about by the inversion of the anteroposterior axis of the limb bud, is thus seen to be a factor of prime importance in the production of reduplications. It is not the only factor, however, for some reduplications have occurred in harmonic combinations, due probably to other disturbances at the time of operation. But quite aside from the latter, it would be wholly unjustifiable to assume that there is any causal relation between the possible utility of the process of reduplication for regulatory purposes and its frequent, or even almost universal, occurrence where it might lead to this result. What seems to be the condition that brings about regulation after the reduplicating bud has arisen is the chance placement of the latter in a position corresponding to that of the normal single limb. This relation is attended by a more advantageous situation with respect to blood and nerve supply than that occupied by the original bud, and in extreme cases this leads to the resorption or suppression of the latter. It is only in such cases that complete regulation takes place and these constitute but about 15 per cent of the total number of reduplications.

In this connection it is important to compare the orthotopic with the heterotopic operations in respect to the regulatory processes just considered. When the limb bud is implanted in abnormal location, functional adaptation does not occur at all, the limbs rarely showing any motor function whatever, unless placed close to the normal position of the limb.[116]

Now the records show (table 8) that the two harmonic combinations in the heterotopic series produced ten single harmonic limbs and twelve reduplications (44.4 and 55.6 per cent, respectively).

TABLE 8

Comparison of heterotopic and orthotopic transplantations with reference to the relative number of single limbs and duplicities in the harmonic and disharmonic combinations

CHARACTER OF COMBINATION	HETEROTOPIC				ORTHOTOPIC			
	Single		Reduplicated		Single		Reduplicated	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Harmonic	10[1]	44.4	12	55.6	24	96.0	1	4.0
Disharmonic	19	86.4	3	13.6	2[2]	3.4	57[3]	96.6

[1] Excluding one anomalous case in which an error of record is probable.

[2] Excluding five cases which became normal single limbs by development of the duplicate and resorption of the original bud, and ten cases which became normal by rotation.

[3] Including five cases in which the original member was resorbed and the single normal limb arose from the reduplicating bud.

In many of the latter, however, the doubling was but slight, involving only the digits. The two disharmonic combinations, on the other hand, produced nineteen single limbs (86.4 per cent) and three (13.6 per cent) reduplications. The results obtained by Detwiler ('18), using much younger limb buds from embryos with open medullary folds, sustain the above results, as far as the harmonic group is concerned. There are eight cases of single limbs and ten reduplications. In the disharmonic group, how-

[116] This has been subjected to a careful analysis by Detwiler ('19, '20), who has shown that the failure to function is not due to lack of peripheral innervation so much as to the insufficient connections within the central nervous system.

ever, there are four normal limbs and three reduplications. If combined with the present results, this would reduce the disproportion somewhat, though it would still leave it very large. There are no cases of complete regulation over to the harmonic position either by rotation or reduplication. Moreover, reduplication in over half the harmonic grafts disturbs the possible harmonic end result.

As pointed out above, the case is very different in the orthotopic operations. Here the harmonic group produced twenty-four out of twenty-five (96 per cent) single limbs, while the disharmonic group produced but twelve single limbs (17.4 per cent), ten of which became harmonic by rotation and are therefore omitted from the tabulation, and fifty-seven reduplications (82.6 per cent), in three of which, however, the primary bud became harmonic by rotation and a possible adaptive result was prevented by the process of reduplication. Leaving out of consideration the cases in which single normal limbs were produced by rotation, there are but two cases left (3.4 per cent) in which the disharmonic relation failed to be followed by reduplication.

In a word, with orthotopic grafts the almost completely dominant factor in producing single limbs or reduplications is the harmony or disharmony of the combination, whereas the case is quite different in the heterotopic grafts, where the harmonic group produced a slight preponderance of reduplications and the disharmonic group a great preponderance of single limbs.

At first sight the above figures might be advanced in support of some hypothesis of an end purpose in development, inasmuch as reduplications are produced in overwhelmingly great number only where they may be taken advantage of to produce an adaptive end result. This, however, would be a hasty conclusion to draw. The orthotopic experiments may be explained as above (p. 106). There the tendency to reduplicate is due first to the disturbance of operation, which, being very slight, is almost always suppressed in the harmonic combinations by the advantage the primary bud has in connecting normally with the surroundings; while in the disharmonic combinations the tendency to reduplicate is not only greatly increased by the reversal of the axis

of the bud, but the primary bud also gains a headway in development that usually cannot be overcome even by the more advantageous position of the reduplicating bud. In the heterotopic operations the frequency of reduplication in the harmonic group may be ascribed to the disturbance due to the operation, together with lack of any special anatomical relations at the seat of implantation that would overcome the tendency to bud by giving the harmonic member a special advantage. All that remains to be accounted for is, therefore, the small proportion of reduplications in the disharmonic group. This should be subjected to further investigation. Standing alone, it can hardly be advanced as an argument for a teleological theory of development.

GENERAL SUMMARY

1. The results given below are based upon the following experiments with the fore-limb bud of the embryo of Amblystoma punctatum:

a. Transplantation to the flank of another embryo posterior to the normal position of the fore limb (heterotopic transplantation), the grafted buds being taken either from the same side of the body (homopleural) or from the opposite (heteropleural), and implanted with the dorsoventral axis upright (dorsodorsal) or inverted (dorsoventral).

b. Transplantation to the normal location of the fore limb after extirpation of the original fore-limb rudiment (orthotopic transplantation), with the same variations as in the heterotopic group.

c. Superposition of one limb bud upon another after removal of the ectodermal covering of the latter, also with the same variations as in the previous groups.

d. Transplantation of half of the circular disc constituting the limb bud, after extirpation of one-half the rudiment. Sixteen different combinations of this experiment (all possible within the limitations imposed) were tried (p. 70).

2. In the early stages of development in any position the transplanted buds give evidence of their constitution by growing out

('pointing') in the direction of what was originally the posterior pole of the anteroposterior axis. Thus, in two of the combinations (homopleural dorsoventral and heteropleural dordosorsal) they point anteriorly or dorsoanteriorly, and in the two others (homopleural dorsodorsal and heteropleural dorsoventral) they point posteriorly or dorsoposteriorly like the normal. In the latter case the subsequent development is usually normal, barring reduplication; in the latter there is a tendency for the limb to stick out to the side and to rotate more or less from the position in which it would be found, were the position determined entirely by the orientation of the bud itself.

3. The palmar surface of the limb tends to develop on the side turned toward the body of the animal, and the ulnar border is dorsal, although the rotation mentioned in the previous paragraph tends to change these positions.

4. The above circumstances determine the asymmetry of the limb as follows: when the dorsoventral axis is not inverted, the original prospective asymmetry persists; when the axis is inverted, the asymmetry is reversed (rules 1 and 2, p. 4). In more general terms: the asymmetry of the limb is determined by two factors, the polarization of the anteroposterior axis of the limb bud and the orientation of the limb bud with respect to the dorsoventral polarization of its organic environment (figs. 2 and 135).

5. In two of the combinations (homopleural dorsodorsal and heteropleural dorsoventral) the asymmetry of the limb which develops corresponds to that of the side of the body on which it is placed (harmonic); in the other two (homopleural dorsoventral and heteropleural dorsodorsal) it corresponds to that of the opposite side (disharmonic).

6. Duplex and multiplex limbs arise frequently from the transplanted buds. They are of all grades and kinds and occur in different proportions in the several experiments. In the heterotopic grafts they are more frequent in the harmonic combinations, while in the orthotopic position they are much more frequent in the disharmonic combinations.

7. In nearly all cases one member of a pair or group can be distinguished as the original (primary) and the other one or ones

as buds. The reduplicating bud is in each case the mirror image of the original, and, when the reduplicating bud is itself doubled, then the one next to the original is the mirror image of the latter, while the one further away is mirrored, with respect to its mate, approximately in accordance with Bateson's rule.

8. Limbs placed in abnormal location, where the specific blood and nerve supply is lacking, are frequently resorbed, and when they do develop, are usually stiff and functionless, or at best show imperfect function. The shoulder-girdle in such limbs is reduced in size and the more outlying elements are lacking.

9. Limb buds placed in normal location (orthotopic) are rarely resorbed and nearly always become functional.

10. Limb buds from the same side of the body normally oriented in orthotopic position develop normally with but slight retardation.

11. When the limb bud from the same side is rotated 180° in its normal location, the results vary considerably, and in the majority of cases reduplications occur. The single limbs are of two kinds, reversed and normal. The former develop in accordance with rule 2 (p. 4), but only one case of this kind has been observed, the others that conform to the rules being reduplicated (rule 3). In the other cases the normal position was reached by the rotation of the limb as a whole about the shoulder-joint. These cases are exceptions to the rules.

12. Of the twenty-seven cases of reduplications in the above group, the original bud grew anteriorly and was reversed. In the three remaining cases the primary limb righted itself by rotation and the reduplicating member was reversed.

13. Regulation by rotation usually takes place when at the operation the limb bud has been rotated anteriorly over the dorsal semicircumference not quite 180°.

14. Limb buds from the opposite side of the body, with the dorsoventral axis normally oriented, produced but one unreversed single limb, in accordance with rule 1 (p. 4), the rest being reduplications (rule 3). In one-sixth of the latter the original bud, which was disharmonic, was resorbed and remained as a small nodule or spur on the reduplicating appendage. The dupli-

cate bud in these cases, having its asymmetry reversed and occupying the right position, became a normally functioning fore limb, perfectly adjusted both functionally and structurally to its organic environment.

15. Limb buds, taken from the opposite side of the body and implanted with the dorsoventral axis inverted, so as to leave the anteroposterior axis in normal relation, formed, with the exception of one reduplication, single limbs, all of which were reversed These limbs were often considerably retarded in development, but, as regards both function and form, they became perfectly adjusted to their new surroundings (rule 2).

16. In the superposed grafts two limb buds are fused into one. In the two harmonic combinations normal single limbs arise. Though at first usually above normal in size, they soon become regulated in this respect. In the disharmonic combinations duplex appendages were formed in a large majority of cases. One case of adjustment by rotation and one case of regulation by reduction of one member of a pair were found.

17. In experiments with half buds there are sixteen combinations possible with the restrictions imposed by the character of the experiment. In addition to the two pairs of attributes of operation common to all of the experiments (*hom.* or *het.*, *dd* or *dv*) there are three others: the bud may be halved vertically or horizontally; the anterior or the dorsal half, or the posterior or the ventral half may be transplanted, the other remaining intact; two like halves or two unlike halves may be united. An analysis of the results shows that no one of these qualities in itself determines the result, but that it is the harmonic or disharmonic character of the combination that determines whether normal or reduplicated appendages arise. Thus, allowing for differences in the number of experiments in each class, 93.4 per cent of the harmonic combinations produced normal limbs, while in the disharmonic groups about that same proportion produced reduplications, of which, however, a considerable number were regulated secondarily through resorption of the disharmonic member.

18. That the limb bud is an equipotential system is shown by the fact that a normal limb may develop after the following oper-

ations, provided the combination is harmonic: 1) extirpation of any half of the bud; 2) fusion of two whole buds; 3) combination of two like halves, the other half being entirely missing; 4) inversion of the limb bud; 5) inoculation of mesoderm cells from the limb under the skin in some other region of the embryo.

19. Except for the circumstance that the dorsoventral differentiation of the limb bud is a function of the orientation of the bud with respect to its organic environment, the limb bud is a highly specific self-differentiating system. Its definitive form must, therefore, be represented in the organic elements (intimate structure) of the limb rudiment.

20. One quality of these elements is their polarization, as shown by the definite relation to the direction of out-growth, assumed by the anteroposterior axis of the limb bud. It is suggested that the asymmetry of the limb rudiment and of other similar systems may be gradually brought about by the change in constitution of the structural elements in a manner similar to the building up of asymmetric molecules in organic compounds.

21. Reduplications are produced as a result of that fundamental attribute of living matter, the power to divide (Bateson). They are induced, in the case of the limb bud especially, by a disharmonic relation between graft and host.

22. There is no fundamental distinction between double supernumerary limbs constituting a symmetrical pair and the single supernumerary symmetrical with the normal one with which it is associated. Bateson's rules may be stated in simplified form in accordance with this conclusion (p. 97).

23. Exceptions to Bateson's rule regarding symmetry relations of supernumerary parts are very rare. Those found in the present study, where two limbs of the same side occurred in linear series, are probably due to the appendages having been far enough apart not to influence one another in development, and at the same time having been under the influence of the same organic environment.

24. Review of the data on regeneration of supernumerary appendages shows that the reversal of asymmetry in one of the members of an enantiomorphic pair is not dependent upon the

reversal of direction of growth, regeneration, or differentiation. The reversed member may grow and differentiate in the same direction as the original, another axis than that on which growth is taking place being the one that is reversed. Reversal may thus occur without axial heteromorphosis and vice versa.

25. In any system, like that of the limb bud at the time of transplantation, in which at least one axis is left undifferentiated, rotation of the elements of which the system is made up might account for reversal. The rotation of cells observed by Hadži and Przibram is, however, concerned primarily with wound healing, and heret is no evidence that it is correlated with the occurrence of axial heteromorphosis or reversal of asymmetry.

26. As an alternative to the hypothesis of rotation, we might consider reversal as due to reversal of molecular asymmetry according to analogy with the behavior of optically active compounds.

27. There is an analogy between the production of enantiomorphic limbs and the production of situs inversus viscerum, as effected by Spemann. Either the reversal may be due to reversal of the intimate structure, or it may take place in spite of the intimate structure through the direct action of mechanical factors on the individual parts of the differentiating system.

28. The transplanted limbs show both regulation of form and functional adaptation. The two often go hand in hand, but not necessarily, for some cases show regulation of form without function, and others functional regulation without form regulation.

29. Functional regulation is largely a matter of innervation, and it occurs only in orthotopic grafts or in those approximately in that position (Detwiler).

30. Form regulation is either primary, as in the case of harmonic combinations, or secondary, as in the disharmonic. In the latter it takes place in one of two ways, either by rotation of the developing limb or by means of reduplication and reduction of the disharmonic member.

31. Form regulation by rotation has been observed to occur only in orthotopic grafts; reduplications in disharmonic combinations are more frequent in orthotopic than in heterotopic

transplantations. While these circumstances lead to an harmonic end-result more frequently where there is functional adaptation as well, this cannot be used as a cogent argument for a teleological theory of development.

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APPENDIX

HISTORIES OF SELECTED INDIVIDUAL CASES

A. Heterotopic transplantations

1. Homopleural, dorsodorsal

Experiment Tr. E. 148. May 4, 1915. Right limb bud to right flank; orientation normal.

May 5. Healed except for two small oval areas.

May 8. Transplanted bud points caudally like normal limb.

May 12. Sketch (fig. 5).

May 15. Transplanted limb slightly bidigitate—not so long or as far differentiated as normal.

May 21. Transplanted limb has grown considerably, normal posture; two digits well marked, third appearing.

May 26. Third and fourth digits show more plainly than in sketch made on May 24 (fig. 6).

June 1. Limb a normal right (homopleural) limb in normal posture (fig. 7).

Experiment Tr. E. 182. April 13, 1916. Right limb bud to right flank; orientation normal.

April 14. Perfectly healed.

April 16. Tranplanted bud as large as normal, points caudally.

April 25. Not so large as normal, points dorsocaudally.

May 1. Much shorter than normal. Points caudally, arising from a ridge-like prominence on side of body.

May 7. Two distinct digits and trace of third. A new bud is growing from base anterolaterally.

May 15. Original limb (the posterior one in fig. 9) has three distinct digits and beginning of fourth, shows elbow bend. It is a normal right (homopleural) in nearly normal posture, though it sticks out stiffly to side. The two limbs are entirely separate from their origin. The anterior member, which is the reduplicating one, shows only a faint trace of the third digit on the anterior border.

Experiment Tr. E. 210. May 10, 1916. Right limb bud to right flank; orientation normal.

May 11. Healed except for small area of uncovered yolk at caudal border of wound.

May 15. Transplanted bud somewhat smaller than normal, points in same direction.

May 18. Posture as of normal right limb. At base a rounded prominence which points headward.

May 22. The prominence has become a reduplicating limb, showing first trace of digits.

May 28. Both limbs well developed. Original is longer and is a right (homopleural) with three digits and faint trace of fourth. Reduplicating member branches from other at elbow and is a mirror image of latter, except that it is less advanced in development.

2. Homopleural, dorsoventral

Experiment Tr. E. 219. May 17, 1916. Right limb bud to right flank, inverted.

May 18. Healing fair; a considerable area anterior to grafted bud not covered with ectoderm.

May 21. Bud looks nearly normal, but points dorso-anteriorly. See sketches made on May 22 (figs. 10 and 11).

May 25. Transplanted bud growing as rapidly as normal. The two point toward each other (figs. 12 and 13).

May 29. Transplanted limb growing well; two distinct digits; points dorso-anteriorly and is apparently a mirror image of normal limb on same side (figs. 14 and 15).

June 5. Rapid growth has continued; limb reaches gills; third digit well marked, fourth beginning to show, elbow bend is slight; hand in an almost vertical plane, palm being anteromedial. Limb is an undoubted left, i.e., reversed (figs. 16 and 17).

Experiment Tr. E. 139. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing perfect, transplanted tissue rather prominent.

May 5. Limb bud points slightly dorso-anteriorly.

May 8. Points distinctly dorso-anteriorly.

May 17. Limb points laterally at angle of a little less than 60° to axis of body. Third digit developing.

May 26. Four digits present (fig. 18). Limb does not look atrophic, but gives no evidence of motility or sensitivity. Position as when last observed. Specimen preserved.

Examination of serial sections shows that the anteromedial surface of the hand is the palm. There can be no doubt, consequently, that the limb is a normal left, having been reversed.

Experiment Tr. E. 140. April 28, 1915. Right limb bud to right flank, inverted.

April 29. Healing of wound good; slight areas still uncovered by ectoderm at ventral border of wound.

May 5. Transplanted tissue growing; but points anterodorsally.

May 11. Digitations plain; limb points as before.

May 17. Limb now points almost transversely, with ulnar digits on anterior border.

May 26. Perfect limb with extensor surface of elbow and ulnar digits dorso-anterior. No motility (fig. 19). Sections of preserved specimen show that ventral surface is palm, and that there can be no question about its being a left limb, i.e., reversed.

Experiment Tr. E. 220. May 17, 1916. Right limb bud to right flank inverted.

May 18. Wound perfectly healed.

May 21. Transplanted bud very prominent. Sticks out to side and slightly anteriorly.

May 25. Transplanted limb points dorso-anteriorly and not much to side. A small reduplicating bud points ventroanteriorly from near base.

May 29. Limb points as before. Two well-marked digits which are slightly irregular. Probably no reduplication.

June 5. Limb sticks out to side almost horizontally; first two digits short, third distinct, fourth barely indicated. Reduplicating hand attached to radial border of the same limb and pointing ventrally has two digits.

N. B. It is uncertain whether this member developed out of the bud noted on May 25.

3. Heteropleural, dorsodorsal

Experiment Tr. E. 227. May 19, 1916. Right limb bud to left side, dorsodorsal.

May 20. Well healed.

May 23. Transplanted bud points dorso-anteriorly and is more massive than normal.

May 26. Grafted limb about same length as normal; points anteriorly and laterally, scarcely dorsally (figs. 20 and 21 drawn one day later).

June 2. Grafted limb now reaches to the axilla of the normal limb; trace of third digit (fig. 22, made day before).

June 5. Limb a normal right (is not reversed), pointing antero-laterally, crossing the normal limb on the medial surface (fig. 23).

Experiment Tr. E. 107. April 9, 1913. Right fore limb to left side, dorsodorsal orientation. Diameter of transplanted bud 3 somites (3 to 5).

April 10. Crescentic uncovered area of yolk ventral to bud, which otherwise is well healed in.

April 14. Grafted bud shows indications of pointing headward.

April 21. Limb growing, pointing anteriorly exactly in reversed position. No digitations yet.

April 28. Limb is clearly a right; third digit now plain, with slight nodule to indicate fourth.

May 5. Possibly slight motility of grafted limb. It is now seen to be a perfect right, sticking out sharply to side, upper arm is inclined dorso-anteriorly, fore arm slightly posteriorly; palmar surface of hand ventro-anterior (figs. 24 and 25).

Experiment Tr. E. 127. May 12, 1914. Right limb bud to left side, dorsodorsal orientation; diameter of bud 3 somites.

May 13. Healing perfect.

May 16. Transplanted bud growing well with slight indication of pointing anteriorly.

May 18. Limb points distinctly anteriorly.

May 22. Two digits show; a reduplicating bud is growing out from base of graft.

June 1. The primary limb now shows fourth digit and is a perfect right (fig. 26, *PR*). The reduplicating member (*DU*) is a left, though not quite so far along in its development. The primary limb now points laterally and posteriorly with its ulnar border headward.

4. Heteropleural, dorsoventral

Experimental Tr. E. 193. April 14, 1916. Right limb to left flank, dorsoventral orientation; diameter of transplanted bud $3\frac{1}{4}$ somites.

April 15. Wound perfectly healed.

April 17. Transplanted bud, rather larger than normal, points posteriorly.

April 21. Transplanted bud has large base, but free portion is slender; points distinctly posteriorly.

April 28. Transplanted bud growing, though much smaller than normal. Points dorsoposteriorly and has two digits.

May 1. Digits more distinct. Slight bending at elbow. Has position of normal left limb.

May 7. Three digits and trace of fourth. A normal left limb in almost normal posture (fig. 27).

Experiment Tr. 167. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Wound completely healed. Transplanted bud prominent.

Feb. 28. Transplanted bud a large blunt prominence which points posteriorly.

March 3. Transplanted bud is very small; may be resorbed.

March 7. Grafted limb much smaller than normal, but growing.

March 11. Considerable growth, but still smaller than normal. Slight indication of elbow bend and digits.

March 15. Digits marked. Limb looks like a normal left reversed, though it sticks out to side more than the normal limb.

March 24. Third digit now plain. Arm points to side. Hand transverse and vertical with respect to body.

March 29. Preserved. Transplanted limb a normal left in nearly normal posture. Fourth digit indicated. Upper arm still sticks out more to side than normal, but otherwise little difference except in size.

Experiment Tr. E. 129. May 13, 1914. Right limb to left side, dorsoventral orientation. Transplanted disc 3 somites in diameter.

May 14. Wound perfectly healed.

May 18. Transplanted bud has grown considerably, points posteriorly.

May 25. Grafted limb points laterally and about 30° caudally, is bidigitate. Hand and perhaps forearm are being reduplicated on anterolateral border.

June 6. Specimen preserved. Imperfectly symmetried double hand, mirrored from radial plane. Arm stretches out to side with elbow bending ventrally. The posterior (original) hand is a left (reversed), as is the arm as a whole; it has four digits normally placed; the anterior (reduplicating) hand is less perfect with only three digits, one long one in the middle and a short one on each side, the first digit being imperfect.

Experiment Tr. E. 163. February 24, 1916. Right limb bud to left flank, dorsoventral orientation.

February 25. Well healed. Very small uncovered area ventral to bud.

February 28. Transplanted bud larger than normal and a little further ventral.

March 3. Bud is growing ventroposteriorly.

March 7. Bud long and slender, growing posteriorly almost on ventral surface of body; probably defective.

March 11. Same.

March 15. Original limb has not changed essentially. A second outgrowth of considerable length arises some distance dorsal to former and projects laterally. Judging by its size, it must have been present and overlooked at last observation.

March 20. Original limb a long bent appendage tapering gradually to a point. New bud has made considerable growth and is bidigitate, the digits spreading more than normally. Indication of elbow bend.

March 28. Secondary appendage has developed a third digit, which, with elbow bend, shows that it is probably a left (reversed).

April 4. Specimen preserved (figs. 33 and 34).. Transverse sections show that limb is undoubtedly a left, since it is the dorsoposterior surface of the hand that is the palm—a very unusual posture. The two limbs have separate girdles which articulate with one another, but are not fused.

Experiment Tr. E. 217. May 17, 1916. Right limb bud to left flank, dorsoventral.

May 18. Perfectly healed.

May 21. Grafted bud almost exactly like normal, points dorsoposteriorly.

May 29. Limb short, sticks out more sharply to side. Digitations will probably be abnormal.

May 31. Possibly symmetrical reduplication of digits (fig. 31).

June 5. Uncertainty about reduplication. One distinct digit (third) on posterior (ulnar) border and also a slight nodule (fourth digit). Prominence on opposite border may not be digits (fig. 32).

June 8. One distinct digit and trace of another formed out of hump on radial side so that hand is now practically symmetrical.

Limb amputated; afterward regenerated; form much like original.

B. Orthotopic transplantations

7. Homopleural, dorsodorsal

Experiment N. E. 3. May 19, 1918. Right limb bud to right side —normal orientation. Pronephros intact.

May 21. Perfectly healed; limb bud normal.

May 25. Both limbs same except that transplanted one is slightly shorter.

May 28. No difference between the two limbs.

June 7. Same. Specimen preserved.

8. Homopleural, dorsoventral

Experiment I. E. 64. May 13, 1916. Left limb bud to left side, inverted. Pronephros removed.

May 15. Small uncovered area ventral to bud, which is larger than normal and slightly posterior to it.

May 18. Limb bud points dorso-anteriorly (fig. 35).

May 24. Transplanted limb points anteriorly into gills. Slight trace of digits; no reduplicating bud (fig. 36, drawn one day earlier).

May 28. Limb points anterolaterally below gills.

May 29. Limb is undoubtedly a right (reversed) and is perfectly normal except as to posture. Ulnar digits appearing on the antero-dorsal border of hand (figs. 37 and 38).

June 1. Transplanted limb sticks out more to side than before and can be brought back further toward normal position. Motility was first observed two days ago.

June 4. Larva has grown rapidly. Transplanted limb functions well; elbow bends toward tail instead of head. Limb a perfect right (figs. 39 to 41, drawn one day later).

Experiment I. E. 60. May 11, 1916. Left limb to left side, inverted. Pronephros removed.

May 12. Well healed, normal-looking bud.

May 15. Grafted bud points dorso-anteriorly.

May 16. Sketches (figs. 42 and 43).

May 18. Beginning of bud like outgrowth (reduplication) posteriorly (fig. 44, *DU*).

May 24. Main limb (*PR*) points more sharply to side and rather more dorsally than normal. Posterior (reduplicating) bud (*DU*) has grown considerably (figs. 45 and 46, drawn May 23).

May 29. Specimen preserved. Limb double from lower part of fore arm. The primary member, which is anterolateral, is a right (reversed), while the other, produced from the posterior bud, is a left (reversed back). Former has short first digit and well-marked third. On the reduplicating hand a trace of third digit present (figs. 47 and 48). Mirror plane is radiodorsal.

Frontal sections show the radii of the two arms are fused at the proximal end, the two ulnae being entirely separate. The humerus is single, i.e., fused throughout and is much more massive than normal. Glenoid cavity is also much larger, but normally oriented. Barring slight abnormalities, the shoulder-girdle seems to be a normal left (not reversed).

Experiment I. E. 63. May 12, 1916. Left limb to left side, inverted. Pronephros removed.

May 13. Fairly well healed.

May 15. Transplanted bud smaller than normal; points dorsally, sharply laterally, and slightly anteriorly (figs. 49 and 50, drawn May 16).

May 19. Points dorso-anteriorly. Slight indication of posterior reduplicating bud at base (figs. 51 and 52).

May 24. Main limb points much more dorsally and laterally than normal (figs. 53 and 54, drawn May 22); no digits. Two reduplicating buds; posterior one (*P.DU*) points as in I. E. 60 (figs. 44 and 45); anterior one (*A.DU*) smaller and spur-like.

May 29. Specimen preserved (fig. 55). Main limb is a right (reversed) with two long digits and third digit on anterodorsal (ulnar) border. The posterior reduplicating member with three digits is free and is in approximately normal position for left limb (reversed back). It is mirrored in a radiodorsal plane. The anterior hand is bidigitate, and is mirrored in an ulnopalmar plane. Arm and fore arm of complex is short and thick.

Experiment I. E. 49. May 14, 1915. Left limb bud to left side of same individual turned 180°. Pronephros removed.

May 15. Fairly well healed; irregular band of uncovered yolk at posterior border of wound.

May 21. Limb bud developing well, points dorsally with very slight anterior inclination.

May 25. Limb points dorsally (fig. 56); two digitations—hand flattened in vertical plane, making angle of 45° with body axis.

May 29. Limb points dorsoposteriorly, as if approaching normal position.

May 30. Limb has rotated still further toward normal position.

June 3. Limb in nearly normal position (fig. 57, drawn June 4).

June 11. Limb tends to lie partially adducted with dorsal surface of manus on bottom.

June 17. Limb now perfectly normal in every respect—form, size, posture, motility (fig. 58, drawn June 21, and fig. 59, drawn from specimen preserved thirty-nine days after operation).

Experiment I. E. 55. May 23, 1915. Left limb bud removed and replaced inverted. Pronephros removed.

May 24. Very well healed. Only a very narrow uncovered strip at posterior border.

May 27. Limb bud smaller and more pointed than normal.

May 30. Limb bud increasing, but not so large as normal. Sticks out more to side.

June 2. Limb bud points dorsoposteriorly, ventral part of bud prominent.

June 7. Limb has grown considerably, points more posteriorly than before. Two digits; manus flattened in transverse plane.

June 11. Posture of limb still abnormal. It points dorsoposterolaterally at about 45° to median and horizontal planes. Trace of third digit.

June 17. Limb normal in every respect—form, size, posture, motility.

Experiment I. E. 85. April 10, 1917. Left limb bud to left side, rotated 180°+ (fig. 60). Pronephros removed; pronephros grafted along with limb bud.

April 11. Well healed.

April 14. Grafted bud about as distinct as normal, points dorsally and slightly anteriorly.

April 16. Bud points dorso-anteriorly ca. 60° to horizontal; attachment slightly posterior to normal. Distinct pronephric swelling ventral to limb.

April 19. Original bud points as before. Posterior reduplicating bud in approximately normal position. Anteriorly, at base of original bud, is a third bud pointing anterolaterally.

April 24. Middle (primary) member points dorsally and has two long digits; anterior member is attached along anterior border of middle; posterior member, in normal posture shows beginning of digits.

May 7. Specimen preserved (fig. 61). Posterior member, an essentially normal left limb from its origin above elbow down, has four digits; it is mirrored from the middle member in a radiodorsal plane. Anterior and middle limbs fused except for distal portion of manus; mirror plane, palmar. The middle member has two long digits and nodules on ulnar border, and is probably a right (reversed). Anterior member has three long digits and ulnar nodule, the third being partly fused with the second of the middle hand. Anterior hand possibly hyperdactylous.

Experiment I. E. 86. April 10, 1917. Left limb bud to left side, rotated 180°— (fig. 60). Pronephros removed; pronephros transplanted with limb tissue.

April 11. Well healed.

April 14. Limb bud prominent, placed a little further posteriorly than normal; points slightly dorsally.

April 16. Transplanted bud points dorsoposteriorly, but more dorsally and slightly more laterally than normal. Pronephric swelling ventroanterior to bud.

April 19. Limb points more dorsally than normal. Small nodule at base is part of pronephros.

April 24. Limb in normal posture not quite so long as normal. Third digit beginning. Reduplicating digit comes off palmar side between first and second digits.

April 29. Second and third digits reduplicated (palmar); limb otherwise very nearly normal. Motility apparently not so good as normal.

May 5. Limb clearly a left (not reversed); arm a little shorter and thicker. Motility now better.

May 7. Specimen preserved (fig. 62).

9. Heteropleural, dorsodorsal

Experiment R. E. 87. May 19, 1915. Right limb to left side, dorsodorsal orientation. Pronephros left intact.

May 21. Small round uncovered area posterior to bud.

May 25. Transplanted bud points anteriorly and laterally (ca. 45°). and is nearly as large as normal (fig. 63, drawn May 26).

May 28. Limb points as before and is growing into gills, one of which is caught in notch between limb and neck and is bent ventrally. Limb itself bent slightly at tip. Digitations faintly indicated.

June 2. Limb thinner than normal and somewhat limp. Bends backward at tip. Two digits only.

June 4. Specimen preserved (fig. 64). The arm sticks out horizontally to side; elbow bends posteriorly; two digits in same horizontal plane with very faint indication of ulnar digits. Limb clearly a right (not reversed), though it is distinctly smaller than normal and is otherwise defective.

Experiment R. E. 70. May 12, 1915. Operation: right limb bud to left side, dorsodorsal orientation. Pronephros left in.

May 13. Still a crescentic area of uncovered yolk at ventral border of bud.

May 15. Transplanted bud more prominent than normal; indications of pointing anteriorly (fig. 67, drawn May 17, at which time the posterior bud could just be made out).

May 21. Points anterolaterally. Attached to base is an almost equally large reduplicating bud, pointing posteriorly and normally located (fig. 68, drawn May 22).

May 27. Both buds growing. The original (anterior) one is still a little further advanced than the other. Figure 69, drawn May 28, shows beginning of digits.

June 7. The original limb is still the larger one, the reduplicated one has two digits and trace of third. The complex has some motility.

June 11. Specimen preserved (fig. 70). The arm is double from the elbow down; the posterior or reduplicating member is not so well developed as the other. The anterior member is a right (not reversed) while the other is mirrored in a radiodorsal plane and occupies a position approximately normal for the left limb.

Experiment R. E. 133. May 25, 1916. Right limb bud to left side dorsodorsal orientation. Pronephros removed.

May 26. Fairly well healed. Small uncovered area ventral to bud.

May 29. Transplanted bud well developed, points dorsoanteriorly about 45°. It is in proper position and about same size as normal.

June 2. Limb points dorso-anteriorly, but more sharply dorsally, extending into notch behind last gill. Two digits show distinctly as in normal limb. Reduplicating bud from posterior border of base has position of normal left.

June 5. Original limb (fig. 75, *PR*) points dorsolaterally toward gills and looks nearly normal. Posterior (reduplicating) bud (*P.DU*) is rather short and arises further tailward than normal limb; digitations beginning. A third small limb (*A.DU*) anterior to the original, projects dorsolaterally, parallel to it.

June 7. Original limb a normal right (not reversed) having two long digits with trace of third and fourth. The posterior bud has grown considerably and is in normal position. Anterior bud shows traces of digits.

June 12. All three limbs have grown and differentiated. Posterior has nodule for third digit and is a normal left (reversed). It is mirrored in a radiodorsal plane while the anterior limb, which is more

nearly parallel to the original, is mirrored in an ulnopalmar plane (fig. 76, drawn June 13).

Experiment R. E. 134. May 25, 1916. Right limb bud to left side, dorsodorsal. Pronephros removed.

May 26. Perfectly healed.

May 29. Transplanted bud, normal size and position, points anterodorsally ca. 45°.

June 2. Limb points dorsally and somewhat laterally. Reduplicating bud on anterior border near base also points dorsally and slightly anteriorly.

June 5. Limb points anteriorly into gills, is short and massive. Three distinct digits, irregular.

June 12. Specimen preserved (fig. 77). Arm short and thick, internal reduplication, certainly from elbow down, mirrored in ulnopalmar plane. The two hands nearly separate; each has two long digits and nodular third. The main limb is a right (not reversed) and the other a left. No posterior reduplication in this case.

Experiment R. E. 69. May 12, 1915. Right limb to left side, dorsodorsal orientation. Pronephros left in.

May 13. Wound well healed. Only a small area at ventroposterior border of wound uncovered.

May 15. Transplanted and normal budsa eqully prominent; former points anteriorly.

May 21. Transplanted limb double. Smaller (originally main) part (fig. 86, *PR*) points anteriorly and is borne upon the posterior portion, *DU*, which is an almost normal looking limb, though smaller than the normal on the opposite side.

May 24. The posterior bud has grown materially and is normal looking with two digits. The anterior bud is relatively much less developed (fig. 87).

May 27. The original bud is reduced to a small spur (*PR*) on the lateral surface of the reduplicating bud, which is almost as large as the normal limb on the opposite side (fig. 88, drawn one day later).

June 3. Transplanted limb perfectly normal. Spur much reduced.

June 7. Spur reduced to a nodule (fig. 89, *PR*).

10. Heteropleural, dorsoventral

Experiment R. E. 80. May 18, 1915. Right limb bud to left side, dorsoventral orientation. Pronephros removed.

May 19. Crescentic band ventroposterior to bud still uncovered.

May 21. Limb bud almost normal in size points posteriorly.

May 24. Limb bud points posteriorly, but more sharply laterally than normal (fig. 93, *TR*).

May 30. Limb points dorsoposteriorly angle of 45°.

June 2. Limb stiff and projects rather more to side than normal; has two digits and slight indication of third on dorsal border (fig. 94, drawn June 1).

June 11. Limb mobile, in normal posture. Brachium shorter than normal. Third digit distinct, fourth indicated.

June 21. Four digits all plain. Upper arm still short (fig. 95 *TR*).

Experiment R. E. 107. May 10, 1916. Right limb bud to left side, dorsoventral orientation. Pronephros out.

May 11. Rather large area of yolk, posterior to graft, still uncovered.

May 15. Transplanted bud points a little more dorsally and more sharply to side than normal.

May 19. Grafted limb (*TR*) about size of normal, though more pointed and projecting more dorsally and laterally (fig. 96, drawn May 18); divided at end (digits or reduplication?).

May 24. Limb on side of operation still projects a little to side. Normal limb seems to move and transplanted one still stiff (figs. 97 and 98, drawn May 23).

May 28. Motility of operated limb still seems deficient. Third digit distinct (fig. 99, drawn May 29).

June 2. Motility better. Specimen preserved.

Experiment R. E. 116. May 16, 1916. Right limb bud to left side, dorsoventral orientation; pronephros removed.

May 17. Perfectly healed.

May 20. Transplanted bud rather small, points slightly dorsoposteriorly.

May 24. Transplanted bud slightly smaller than normal and points a little more to side. Figure 100, drawn on May 23, shows scarcely any difference between the two buds.

May 28. Transplanted limb (*TR*) practically exact counterpart of normal (figs. 101 and 102, drawn May 29).

May 31. Transplanted limb has third and trace of fourth digit. Function normal.

Experiment R. E. 93. May 19, 1915. Right limb bud to left side, dorsoventral orientation; pronephros mostly removed.

May 21. Well healed; bud smooth and mound-like.

May 25. Transplanted bud points dorsoposteriorly with a secondary bud at anterior border of base.

May 28. Posterior bud (*PR*) is normal in position and orientation, and is even a little longer than the normal limb on the opposite side. The other (anterior) nodule (*DU*) is growing into a limb and also points posteriorly (figs. 103 and 104).

May 30. Posterior bud growing more rapidly than other; digitations plain. Anterior bud sticks out more to side, shows trace of digitations.

June 4. Two hands, each with two digits; some excrescences. Arm a little shorter than normal. The posterior bud is (probably) a left, and its position is approximately normal. The other is mirrored from its dorsal surface. Both hands have faint trace of third digit. Excrescences which seem to involve mainly the skin make an exact interpretation of this case difficult (fig. 105).

C. Superposed transplantations

13. Homopleural, dorsodorsal

Experiment S. E. 3. April 20, 1916. Right limb bud to right side, normal orientation; mesoderm of host torn in posterior part of wound, otherwise intact.

April 21. Well healed; limb bud considerably larger than normal.

April 24. On operated side limb bud larger than normal.

April 26. Operated limb slightly larger, otherwise no difference.

May 2. Operated limb still slightly larger.

May 8. The two limbs exactly alike (fig. 106).

14. Homopleural, dorsoventral

Experiment S. E. 18. April 13, 1917. Right limb bud to right side, inverted; mesoderm all left in.

April 14. Perfectly healed.

April 17. Operated limb bud sticks out more to the side, and is more massive than normal.

April 21. Operated limb double; posterior component more massive, occupies normal position; other is attached to radial border and points dorsoposteriorly; neither has digits.

April 25. Reduplication of hand and part of fore arm; one component is in approximately normal position, the other sticks out to side.

April 30. Limb has grown; relations essentially the same as before.

May 1. Specimen preserved. The main limb projects horizontally to side and is inclined about 30° to the transverse plane; palm is ventral, ulnar border posterior. The reduplicating member with two digits is attached to the radial border of the wrist, pointing anteriorly and laterally (fig. 107).

Experiment S. E. 10. May 26, 1916. Right limb bud to right side inverted; mesoderm of host torn along posterior border of wound and a little tissue lost.

May 27. Perfectly healed.

May 30. Limb on operated side points a little more dorsally than normal. Ventro-anterior to main bud another fairly prominent mass of tissue (like S. E. 9, in which an extra digit developed).

June 4. Limb on operated side considerably smaller than normal, and still points more dorsally; digits just beginning.

June 12. Operated limb still less developed than the unoperated; third and fourth digits beginning to show.

June 15. Practically no difference between the two limbs; the operated one is possibly a little thicker.

15. Heteropleural, dorsodorsal

Experiment S. E. 12. May 26, 1916. Left limb bud to right side, dorsodorsal orientation; mesoderm of host not injured at all.

May 27. Perfectly healed.

May 30. Limb on operated side (*TR*) more massive than normal and points dorsally; on posterior border at base there is a small rounded prominence or bud (figs. 110 and 111, *HOM*, drawn May 31).

June 4. Main limb (*HET*) is spindling and points laterally, though inclined slightly dorsally and posteriorly; there are three imperfectly marked digits at the tip. From the bud at the posterior border there has developed a second limb (*HOM*) which has normal posture; it is of considerable size and shows beginning of digitations (fig. 112, drawn June 5).

June 7. The posterior member is much more massive than the other, which still sticks out to side and shows a very imperfect hand.

June 19. The posterior member (*HOM*) is practically normal with four digits; it is mobile, though probably there is some extensor weakness of hand. The anterior member (*HET*), a left (not reversed), is thin and atrophic, the imperfect hand having three digits. It arises from near the shoulder of the other, the reduplicating plane being approximately radial (fig. 113, drawn June 12).

Experiment S. E. 6. April 21, 1916. Right limb bud to left side, dorsodorsal orientation.

April 22. Perfectly healed.

April 26. On operated side there are two projections in limb region (fig. 109 *A*).

May 2. Operated limb not quite so advanced as the normal; distinct spur (fig. 109 *B*, *S*) on radial border, probably from the anterior of the two prominences.

May 8. Specimen preserved (fig. 109). Operated limb (*TR*) not so advanced as normal, digits not so well developed. The spur (*S*) is attached to the antero-lateral border of arm above elbow and is as large as one of the primary digits. It is a radial reduplication which has remained abortive.

16. Heteropleural, dorsoventral

Experiment S. E. 11. May 26, 1916. Left limb bud to right side, dorsoventral orientation; mesoderm torn along posterior border of wound; no tissue lost.

May 27. Perfectly healed.

May 30. On side of operation a large limb bud points dorsoposteriorly like the normal. Ventrally another distinct but smaller bud (*DU*) points ventroposteriorly (figs. 114 and 115 drawn May 31).

June 4. Operated limb (*TR*) longer and further advanced (digits) than normal (*N*). Otherwise no abnormality (fig. 116, drawn June 5). No trace of ventral bud noted last time.

June 7. Limb on operated side still a little larger than the other.

June 12. Still some difference in size (fig. 118); limb normal in form; motility not so good as normal, extensors of hand weak.

June 19. Function of operated limb very much better, almost, if not quite, normal. Specimen preserved. No difference in size of limbs.

D. Transplantation of half-buds

18. Homopleural, dorsodorsal

Experiment H. E. 6. April 12, 1917. Anterior half of limb bud to anterior half, normal orientation (fig. 120, *1*). Operation on right side. Pronephros removed and transplanted.

April 13. Perfectly healed.
April 17. Limb bud on operated side normal.
April 20. Same.
April 30. Same.

19. Homopleural, dorsoventral

Experimental H. E. 31. April 9, 1918. Posterior half of right limb (inverted) in place of anterior right (fig. 120, *6*).

April 10. Wound perfectly healed.

April 15. Operated limb bud smaller than normal, and stands out more sharply from body. It is more distinctly marked off (points) anteriorly.

April 18. Operated bud distinctly more massive (fig. 121, *TR*).

April 21. Hand double, but coalesced to end. Digitations indistinct.

April 30. Practically a normal right limb with an accessory hand growing from back of hand. The preserved specimen shows this to be a case of reduplication mirrored from a dorsal plane slightly inclined to the radial. The reduplicating member consists of second and third digits, (*2′* and *3′*) the former slightly bifurcated. The first (radial) digit is not doubled (fig. 122).

Experiment H. E. 29. April 9, 1918. Ventral half (inverted) of right limb bud in place of dorsal right (fig. 120, *8*). Embryo from which graft was taken, stained in Nile-blue sulphate.

April 10. Healing good. Small round area still uncovered by ectoderm at ventroposterior border of wound.

April 15. Operated bud has grown considerably, pointing dorsally, and slightly anteriorly. Whole free portion of bud covered by stained (grafted) ectoderm.

April 18. Limb on operated side points dorsoposteriorly with reduplicating nodule on posterior border.

April 21. Limb short; double hand.

April 26. Well-developed double hand. Anterior member further developed, sticks out to side; is a left; palm anterior; other a right; dorsoradial reduplication.

May 4. Almost perfectly symmetrical double hand.

May 6. Specimen preserved. Arm as a whole a right, as is the posterior hand. This has three digits and a nodule for fourth, the first

two digits being partly syndactylous. Reduplication is radial. The anterior hand (which is a left) has two long digits and a well-developed third.

Experiment H. E. 2. April 18, 1916. Dorsal half of right limb bud (inverted) in place of ventral right (fig. 120, *7*).

April 19. Healing good, but a groove dividing the limb region horizontally still indicates line of suture.

April 21. Two rather distinct humps on operated side; larger one ventro-anterior.

April 26. Operated limb bud nearly normal, but there is a bud-like projection on the anterolateral border (fig. 123, *S*).

May 1. Transplanted limb larger than normal. The anterior process is now a spur at elbow of the main limb, which is nearly normal.

May 8. Preserved. The operated limb, especially above the elbow is a little thicker, but is otherwise normal. The spur is a nodule just above elbow on radial border (fig. 124).

Experiment H. E. 5. April 12, 1910. Posterior half of right limb bud (inverted) in place of anterior right (fig. 120, *6*).

April 13. Perfectly healed except for minute uncovered area at dorsal border.

April 17. Operated limb bud double; large posterior bud points normally, but is not so large as normal; anteroventral bud is much smaller; it is clear that former is developing out of the remaining half of the limb bud of the host, while the latter is from the graft.

April 20. Both buds have grown; posterior one points more dorsolaterally than normal and shows first beginnings of digitations; anterior bud prominent, rounded, no digitations; the two are separate to base.

April 25. Posterior member is fan-shaped, with three long digits, and beginning of ulnar digits on each margin; anterior member much shorter, with faint indication of digitations.

April 30. Posterior member has a symmetrical fan-shaped five-digitate hand; as a whole it is a right limb, as indicated by elbow bend. Anterior member much shorter and thicker.

May 21. Specimen preserved. The posterior member has a double hand, of which the posterior is a right and the anterior a left. The anterior member is a nearly normal right, with the hand a little twisted and syndactylous second and third digits (fig. 125). This is an anomalous case.

Experiment H. E. 13. May 3, 1917. Posterior half of right limb bud (inverted) in place of anterior half (fig. 120, *6*).

May 4. Perfectly healed.

May 7. Two distinct though small limb buds, one ventro-anterior, the other dorsoposterior.

May 10. Two buds united at base; both project sharply laterally and slightly posteriorly.

May 14. Two limbs; bifurcation in Y-form about at elbow. Anterior member shows two digits; posterior, none.

May 21. Specimen preserved (fig. 126). Hand double; limb (*TR*) as a whole looks like a right, including the anterior hand (*HOM*), which has two long digits and a distinct third. The posterior hand (*HET*) is a left, also with three digits, the first of which is truncated. Reduplication ulnar.

20. Heteropleural, dorsodorsal

Experiment H. R. E. 1. April 18, 1916. Posterior half of right limb bud in place of anterior left (fig. 120, *10*).

April 19. Well healed.

April 21. Two distinct humps on operated side; dorsal one larger, ventro-anterior one quite small.

April 26. Limb on operated side about as large as normal, but projects laterally.

May 1. Reduplication of hand; three digits, fan-like.

May 8. Specimen preserved (fig. 127). Arm somewhat thicker than normal, projects posteriolaterally with hand flexed ventrally. Hand symmetrical, with five digits, of which middle one (*1*) is defective. The posterior half of the hand (*HOM*) is a left, the anterior (*HET*) a right.

Experiment H. R. E. 5. April 19, 1916. Dorsal half of right limb bud in place of dorsal left (fig. 120, *11*).

April 20. Perfectly healed.

April 24. Operated limb bud not quite so large as normal, points laterally and slightly anteriorly.

April 29. Operated limb double (fig. 128).

May 8. Specimen preserved (fig. 129). The dorsal (posterior) member (*HOM*) is a nearly normal left arm; first digit short; third is small, and fourth not visible. The ventral (anterior) member (*HET*) arises just below elbow, and likewise shows three digits, of which first is short; plane of mirroring is radial.

Experiment H. R. E. 11. April 11, 1917. Anterior half of left limb bud in place of posterior right (fig. 120, *9*). Wound perfectly healed same evening.

April 14. Operated limb bud very large and prominent, position normal.

April 16. Limb bud massive, points dorsolaterally; almost perfect anteroposterior symmetry.

April 23. Limb has now assumed normal position; first two digitations show; a distinct but small spur at elbow on radial border points laterally.

May 4. Operated limb normal; spur much reduced.

May 7. Specimen preserved. Arm normal; spur a mere nodule about middle of upper arm, radiodorsal border (fig. 130).

Experiment H. R. E. 43. April 5, 1918. Anterior half of left limb bud in place of posterior right (fig. 120, *9*).

April 6. Perfectly healed.

April 9. Operated limb a little more massive than normal.

April 11. Limb more massive, rounded, not more distinctly marked off posteriorly than anteriorly when viewed from above. Points dorsally slightly posteriorly in lateral view.

April 14. More massive, but otherwise normal.

April 22. Not quite so long; syndactyly first two digits, otherwise normal.

May 4. Normal except for syndactyly.

Experiment H. R. E. 9. April 11, 1917. Posterior half left limb bud in place of anterior right (fig. 120, *10*). Wound perfectly healed same evening.

April 14. Operated limb bud looks nearly normal. No definite pointing.

April 16. Two rather distinct nodules, the posterior one considerably more prominent.

April 18. Posterior bud smaller than normal, points dorsolaterally. Anterior one not so definite.

April 23. Two entirely separate limbs; anterior one is shorter, points ventroposteriorly; posterior one points straight to side; no digitations.

April 28. Two limbs point posterolaterally, parallel to one another; anterior one thicker, has two digits and beginning of third; apparently a normal, though much smaller, right limb in approximately normal position. Posterior limb thinner, rod-like, with no digits.

May 4. Anterior limb a normal right; posterior, very imperfect, has one long digit and third digit nodule on upper border; elbow bend shows; limb probably also a right (?)

May 12. Anterior limb has good function.

May 21. Specimen preserved. The anterior member is a normal right, somewhat smaller than the unoperated limb. Posterior member has one long and one short digit, and has same general position as the anterior. Total view leaves in uncertainty which is palmar surface (fig. 131). Sections show clearly that the ventrolateral surface is the palm and that the limb is therefore a left.

Experiment H. R. E. 20. May 2, 1917. Dorsal half left limb bud in place of dorsal right (fig. 120, *11*).

May 3. Wound perfectly healed.

May 7. Operated limb about full size, points anteriorly and slightly dorsally.

May 10. Projects straight to side. Slight indication of posterior reduplicating bud from near attachment of limb.

May 14. Main limb points dorsolaterally and slightly posteriorly. The reduplicating bud is attached near base and in part directly to body wall; it has grown considerably, but is still without digits.

May 18. Main (anterior) member sticks out to side, but now points distinctly posteriorly as well, is slender and with two digits. Other limb shorter, but considerably stouter, with faint indication of two digits.

May 27. Anterior member has remained slender, and still has but two digits; distinct elbow bend; general shape indicates it to be a right. The posterior member, practically normal except slightly underdeveloped, is an undoubted right; first two digits partly syndactylous (first short); third is distinct and fourth indicated. Some function in this member.

May 28. Specimen preserved; form of limbs shown in figure 132. Sections show that there are two separate sockets in the shoulder-girdle for the two limbs. The medial surface of both is the palm. Hence both are rights.

21. Heteropleural, dorsoventral

Experiment H. R. E. 36. April 4, 1918. Ventral half left limb bud (inverted) in place of dorsal right (fig. 120, *16*). Pronephros removed.

April 5. Perfectly healed.

April 8. Operated bud a little sharper; points normally (dorso-posteriorly).

April 10. Nearly normal; points slightly more dorsally.

April 13. Normal.

April 16. Normal limb; digitations show.

April 30. Specimen preserved (fig. 133).

Experiment H. R. E. 10. April 11, 1917. Anterior half left limb bud (inverted) in place of anterior right (fig. 120, *13*). Wound completely healed same evening.

April 14. Limb bud operated side nearly normal looking.

April 16. Almost perfectly normal.

April 18. Operated limb normal except slightly larger.

April 23. Reduplication of digits radial side.

April 28. Operated limb a normal right with two digits arising from palmar surface near radial border; arm somewhat thicker than normal.

May 7. Specimen preserved (fig. 134). The first digit is somewhat small; the reduplicating digits (*DU*) are united at base.

[Reprinted from the Proceedings of the National Academy of Sciences
Vol. 7, No. 1, pp. 41–44, January 1921]

THE PRESENT STATUS OF THE LONG-CONTINUED PEDIGREE CULTURE OF PARAMECIUM AURELIA AT YALE UNIVERSITY

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Communicated by R. G. Harrison, December 30, 1920

As a matter of record it seems advisable to bring up to date and summarize the chief results derived from the writer's main pedigree culture of Paramecium aurelia.

This culture, designated Culture I, was started on May 1, 1907, by the isolation of a "wild" individual which was found in a laboratory aquarium.[1] The original specimen was placed in about five drops of culture fluid on a glass slide having a central ground concavity, and when this animal had produced four individuals, each of these was isolated on a separate slide to form the four lines of the culture. The four lines have not been kept distinct from one another throughout the work, but have been replenished by cells from one of the sister lines when, through accident or otherwise, one or another of the lines has become extinct.

The culture has been maintained by the isolation of a specimen from each of these lines practically every day, with the exception noted below. The number of divisions in each line has been recorded at the time of isolation and the average rate of these four lines has again been averaged for varying numbers of days (5, 10, or 30 days) as the exigencies of the different experiments demanded. These data have afforded the graphs of the division rate. Permanent preparations have been preserved from time to time for the study of the cytological changes during the life history. During the first eight months of the work the culture medium consisted of infusions of hay and fresh grass, but from February, 1908, to the present time various materials collected from ponds, swamps, etc., have been employed. The infusions were thoroughly boiled to prevent the contamination of the pure culture with foreign strains of Paramecium. In short, the cells of the four lines of the culture to-day are direct lineal descendants by division of the single animal isolated in 1907.

The object of starting the culture was to determine whether Paramecium can reproduce by division indefinitely without recourse to conjugation. Throughout the work the possibility of conjugation in the four lines of the culture has been precluded by the almost daily isolation of the products of division. Accordingly its continued life and health has long since justified the conclusion that conjugation, involving syncaryon formation, is not, as previously generally maintained, a sine qua non for the continued life of Paramecium, in particular, and, presumably, of Infusoria in general. At the completion of the

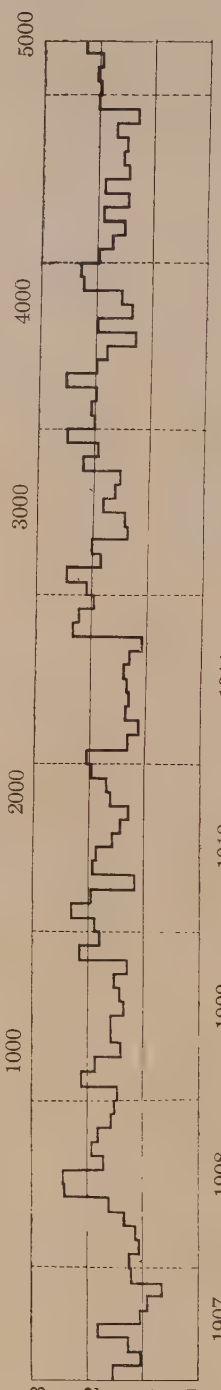

FIG. 1

Graph showing the rate of reproduction of the pedigree race (I) of Paramecium aurelia during the first eight years of culture. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for each month. The vertical broken lines indicate the limits of the calendar years. The figures 1000, 2000, etc., represent generations and are placed above the periods in which they were attained.

culture's first five years of life, during which over 3029 generations were attained, the writer stated that "the organisms of the present generation are in as normal morphological and physiological condition as the original 'wild' individual isolated to initiate the culture. This study has indicated that, under favorable environmental conditions, the protoplasm of the cell originally isolated possessed (at least) the potentiality to produce similar cells to the number represented by 2 raised to the 3,029th power, or a volume of protoplasm not less than 10^{1000} times the volume of the Earth. I believe this result indicates beyond question that the protoplasm of a single cell may be self-sufficient to reproduce itself indefinitely, under favorable environmental conditions, without recourse to conjugation. . . . . "

As a matter of fact organisms taken from this culture of Paramecium have shown but little tendency to conjugate. From time to time experiments to induce conjugation have been carried out in mass cultures started from the pedigree lines. The first successful experiment occurred in December, 1913, at about the 4100th generation.[2] The next epidemic of conjugation in mass culture occurred June, 1920.

Careful studies of the rate of division of sub-lines derived from the main lines and bred under the most constant environmental conditions revealed the fact that there are inherent, normal, minor, periodic rises and falls of the fission rate due to some unknown factor in cell phenomena. These were termed Rhythms.[3] In a search for the underlying factors of rhythms a complicated internal nuclear reorganization process was discovered and named Endomixis.[4] This process involves the periodic formation of a complete new nuclear apparatus by a definite sequence of normal morphological changes which simulate those occurring in conjugation. Endomixis, in essence, consists of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micronuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new macronuclear and micronuclear apparatus of the cell. This results in the

YEAR	GENERATIONS	DAILY AVERAGE DIVISION RATE
1	452	1.24
2	690	1.89
3	613	1.68
4	612	1.67
5	662	1.81
6	692	1.89
7	671	1.84
8	679	1.86
Average for 8 yrs.	634	1.74

reorganization of the cell (endomixis) without the coöperation of two animals involving syncaryon formation (amphimixis) as in conjugation.

After endomixis was worked out, it seemed unnecessary to continue longer the main pedigreed culture and accordingly on May 1, 1915, at the 5071st generation, the experiment was *formally* considered as closed.

The record of divisions for each year of the life of the culture to May 1, 1915, may be tabulated as shown in the preceding table.

Thus for eight years the culture averaged about fifty generations per month. However, at the formal termination of the experiments in 1915, I was reluctant to discard the race and was tempted to keep it under control but without such exacting daily observation and recording of the daily division rate. In this manner it has been maintained up to the present (December, 1920). But, from time to time, thirty-day tests have been made of the vitality of the race under the former rigid culture conditions, and in each case the same general average division rate has been revealed as during the first eight years of its life. For example, during December, 1915, 52 generations; during December, 1917, 56 generations; and during November, 1920, 54 generations were attained. On the basis of such tests it is fair to make the exceedingly conservative estimate of 600 generations attained each year since May 1, 1915. This would give, in round numbers, 8400 generations attained by the culture during the $13^1/_2$ years of its life to date.

Thus the conclusion is still justified that conjugation is not a necessary phenomenon in the life history of Paramecium aurelia under favorable environmental conditions. But there is an internal reorganization process (endomixis) which occurs periodically. Whether endomixis is a necessary factor for the continuance of the race is another question—a new question which has been raised by these studies, and is now under investigation.[5]

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[2] Woodruff, L. L., "So-called conjugating a non-conjugating races of Paramecium," *J. Exper. Zool.*, **16**, 1914.

[3] Woodruff, L. L. and Bai sell, G. A., "Rhythms in the reproductive activity of Infusoria," *J. Exper. Zool.*, **11**, 1911; "The temperature coefficient of the rate of reproduction of Paramecium aurelia," *Amer. J. Physiol.*, **29**, 1911.

[4] Woodruff, L. L., and Erdmann, R., "A normal periodic reorganization process without cell fusion in Paramecium," *J. Exper. Zool.*, **17**, 1914.

[5] Woodruff, L. L., "The problem of rejuvenescence in protozoa," *Biochem. Bull.*, **4**, 1915; "The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia," *Biol. Bull.*, **33**, 1917.

AUTHORS' ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 6

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 32, No. 2, February, 1921

STUDIES ON THE RETINA

THE STRUCTURE OF THE RETINA OF ALLIGATOR MISSISSIPPIENSIS AND ITS PHOTOMECHANICAL CHANGES

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THIRTEEN FIGURES

INTRODUCTION

Owing to the variation in kind and distribution of the visual cells, the reptilian retina offers an interesting field of investigation in structure and function, with particular reference to the probable functions of the rods and cones under diurnal and nocturnal conditions (duplicity theory). Detwiler ('16) studied the structure and photomechanical changes in the retina of a number of turtles and of lizards, and with the idea of making another contribution to our knowledge of the conditions holding in the reptiles the present investigation was undertaken. The work was started in the spring of 1917, but was necessarily abandoned, and only recently has it been possible to resume it.

The literature on the crocodilian eye is small and rather unsatisfactory. Heinemann ('77), in a study of the eyes of Mexican reptiles, described the visual cells of Crocodilus rhombifer cuv. as consisting of characteristic rods alternating with considerably fewer and shorter cones. In addition to the typical rods he found a much less numerous kind, with very long outer segments of platelet structure. The cones are described as being also of two kinds, thick and slender, neither of which contain colored oil drops, and which occur singly and together to form double cones. There is an ellipsoid with a small central body in the peripheral portion of the inner segment of both and

a paraboloid in the slender cones. He could distinguish the cone nuclei from the rod nuclei by their more spherical form and larger size, but describes both as occupying a single layer.

Tafani ('83) studied the retina of the crocodile, Champsia lucius, and found rods to be the predominant type of visual cell. In the anterior part of the retina there are practically no cones, but as the fovea is approached they become gradually more numerous than the rods. He found, unlike Heinemann, only one kind of rod. He describes the cones as being short, with a barrel-shaped inner segment, but with an outer segment similar to that of the rods, although his figures do not bear out his description. The differences between the nuclei of the cones and of the rods, which occur in a single layer, he considers too slight and inconstant to be considered as of any significance.

Chievitz ('89) described in some detail the pigmented epithelium and the tapetum. In the eye of Alligator mississippiensis the tapetum extends through the entire upper half of the retina in the form of a bright band, reaching nearly to the ora, while its lower margin lies about 2 mm. above the entrance of the optic nerve in a 32-cm. specimen. In this bright band he found a fovea in the form of a very superficial, narrow furrow with thickened edges, and running horizontally across the entire tapetum about 1 mm. from its lower edge. He could not decide whether rods as well as cones occurred. In a vertical section of the eye of Crocodilus intermedius the tapetum is seen as a longitudinal bright stripe in the middle of the pigmented epithelium. In this region the middle part of the epithelial cells contain a number of fine, whitish, opaque granules of guanin, which when removed leave the middle portion of the cells colorless, while the choroidal and the vitreal portions contain melanin. The nucleus lies in the guanin-containing portion, directly against the basal pigment. At the margin of the tapetum black pigment is present in almost the entire cell; toward the middle, the vitreal pigment is gradually reduced and eventually exists only in the form of isolated, iregularly distributed small masses, between which the guanin comes to the edge of the cells. In the alligator the pigment in the choroidal portion of the cells is sparse and

the nuclei are very close to the basal cell boundary. The pigment processes reach as far as the inner segments of the visual cells, the outer segments being deeply imbedded in the guanin-containing portion of the epithelium.

Krause ('93) described the visual cells in the retina of Alligator mississippiensis and quotes from Hofmann's description of the retina of Crocodilus vulgaris. In the alligator, Krause considers that the rods could be taken for small cones, because the slender inner segments are slightly tapering, while the outer segments are almost cylindrical. The inner segments of the cones, on the other hand, are thick and the outer segments short and pointed. Hofmann describes the rods of the crocodile as numerous except in the fovea and the surrounding regions of the retina. They are very similar to the red rods of the frog, and Krause reproduces a figure from Hofmann of a rod and cone. The cones are single and double. Krause reproduces (again from Hofmann) cones with very long, pointed outer segments from the fovea. There are no rods in the area and only single cones, the inner segments of which become narrower as the fovea is approached. According to Krause, the nuclei of the visual cells in Alligator mississippiensis are all in one row, the cone nuclei being rounder than those of the rods. In the crocodile, Hofmann says that they occupy two rows, with the rod nuclei next to the external limiting membrane.

Abelsdorff ('98) considers that very strong support is given by the conditions in the reptilian retina to the view first put forth by Max Schultze, that the rods serve for the reception of weak and colorless light stimuli. He calls attention to the fact that most reptiles have practically only cones, the exceptions being the geckos (in some of which the cones seem to be entirely lacking), the crocodiles, and the boa. These are all nocturnal animals. The crocodile, he says, on account of its rod-rich retina, is not only capable of seeing in a very weak light, but can find its way about in pitch darkness, this property being enhanced by the light reflecting tapetum in the upper portion of the eye, the rods being thereby doubly stimulated. Abelsdorff points out that it is particularly in water that the upper part of the eye

needs an increase in intensity of the light impression more than does the lower part of the eye, because the upper portion receives only what little light may be reflected from the bottom.

He figures the tapetum in a sagittal section where it can be seen in the upper portion of the eye between the choroid and retina proper, going over in the lower portion of the eye, with a gradually increasingly thick black border, into the guanin-free, melanin-containing portion of the pigmented epithelium. Attempts to demonstrate a change in position of this pigment in light and darkness were unsuccessful. It is interesting to note in this connection that Garten ('07, p. 89) considered it worth while to have this experiment repeated, which he did, with results (p. 108) substantiating those of Abelsdorff. Abelsdorff describes the rods of the alligator as being similar to those of frogs, but of narrower diameter. He found that the visual purple, investigated by direct observation of the opened eye as well as ophthalmologically (assisted therein by the presence of the tapetum), was not confined to the upper portion of the eye, but, by turning the retina over and looking at it from the visual cell side, could be seen as well in the lower portion. He investigated the bleaching of the purple in daylight as well as the relative amount of bleaching and the time relations upon exposure to light of various wave length. The fact that the purple was seen throughout the eye would seem to indicate that the rods occurred throughout the retina, although, if one chose to follow Edridge-Green, it might be assumed that the purple diffused into the regions where the rods were not present.

Finally, Garten ('07, p. 109) describes the visual cells in Alligator lucius. In the upper part of the retina (guanin portion) there are large cylindrical rods only, which are surrounded in the light as well as in the dark eye by a mantle of guanin. This part of the retina is absolutely cone free. In the lower portion of the retina the visual cells are relatively small, possessing a very thin tapering outer segment, which in light as well as in dark eyes is buried in pigment. Garten considers these to be all cones. He refers to Abelsdorff as having described visual purple in the lower portion of the retina, and thinks, since he

(Garten) has shown that there are only cones in this region, that this matter should be reinvestigated.

The conditions in the alligator eye Garten uses in substantiation of his conception of the functional value of the migration of pigment in connection with the movement of the visual cells. Since he finds the two parts of the retina containing exclusively cones or rods, he argues that pigment migration therefore should not take place, because the perceiving power of the eye would not thereby be in anyway enhanced. Garten calls attention to the importance of the fact that the conical visual cells go over very gradually into those of rod form, and points out that it is exactly at this transition place that Chievitz localized the fovea.

In two general reviews by Pütter ('12) and Franz ('13) the conditions found in the crocodilian retina are summarily given, but no new matter contributed. It should be recalled that Pütter is of the opinion that, although some of the elements in the reptilian retina may be cylindrical in form (that is rod-like), they are all nevertheless to be regarded functionally as cones, on account of their dendritic mode of connection with the bipolar cells.

METHODS

The alligators, which were between 45 and 55 cm. long, were treated as follows. Two animals were placed in a dark room for twenty-four hours, at the expiration of which time one of them was removed to bright diffuse daylight for seven hours. At the end of this time both were killed. The upper jaw, with the eyes, was removed with a pair of large bone forceps, bisected, and dropped into a large dish containing an abundance of Perenyi's fluid. The time required for this operation did not exceed thirty seconds, and in the dark was carried out in the weak light from a photographic lamp. The pupil of the alligator is vertical, and when the animals are placed in light the aperture remaining after a few seconds' exposure is but a mere slit. The pupillomotor reaction is so decisive, characteristic, and easily measurable, that experiments have been begun on the relative efficiency of spectral lights upon it. These will be reported later.

The halves of the upper jaw containing the eyes were allowed to remain in the fixing fluid for an hour, in light or darkness, respectively, without being disturbed. After the expiration of this time, they were carefully dissected out and dropped into

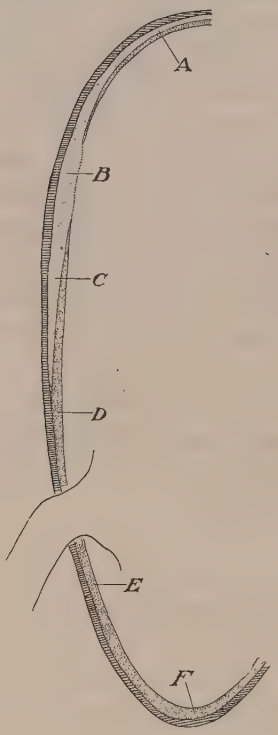

Fig. 1 Diagrammatic drawing of sagittal section of the epithelial pigment layer. The guanin is indicated by the lightly stippled, the melanin by the heavily stippled area; the choroid is shown by horizontal lines. × 11.

fresh fixative, where they remained from four to five hours longer. Sagittal and horizontal sections 10μ thick were made, stained in eosin and toluidin blue, iron haematoxylin and eosin, or in Ehrlich's haematoxylin and eosin. All methods yielded good results.

ANATOMICAL

Epithelial pigment layer. A retinal tapetum occurs in the dorsal and posterior portions of the retina to within 1.5 mm. of the entrance of the optic nerve. It is formed by the inclusion of guanin in the cells of the epithelial layer. The relative amount and distribution of the guanin and the ordinary melanin is shown in figure 1. In the region designated by B the epithelium is relatively devoid of melanin, which forms a narrow border of a

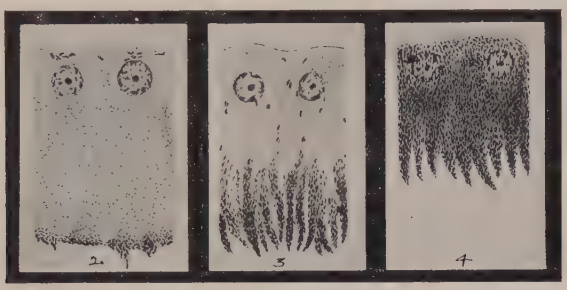

Fig. 2 A portion of the tapetum designated by the letter B (fig. 1), showing broad zone of guanin, a narrow vitreal border of melanin, and a few scattered needles of pigment near the choroidal margin. × 665.

Fig. 3 A portion of the epithelial layer designated by letter C (fig. 1), showing choroidal guanin-containing portion and vitreal melanin-containing portion. × 665.

Fig. 4 A portion of the epithelial pigment layer corresponding to the region designated by letter D (fig. 1). × 665.

few needles along the vitreal margin and occurs also as scattered needles here and there in the choroidal portion of the cells (fig. 2). As the optic nerve is approached, the amount of melanin gradually increases as the guanin decreases, until within about 1.5 mm. above the entrance of the optic nerve guanin is no longer found. The gradual increase in the amount of melanin as the optic nerve is approached is seen in figures 2, 3, and 4, which show in detail the condition as found at the levels B, C, and D in figure 1. Above the level B in figure 1 the guanin again gradually decreases in amount and the melanin shows a corresponding increase (level A, fig. 1).

The guanin is light grayish-brown in color (stained sections), finely granular and fairly uniformly distributed through the cell (fig. 2). In the tapetum (level B, fig. 1), the guanin-containing protoplasm, although covering over the outer segments of the visual cells, does not show the finger-like projections which so typify the melanin-containing portions of the epithelium (figs. 2, 3, and 4).

In the lower portion of the retina the epithelial layer is entirely devoid of guanin. Here the melanin, in the form of delicate brownish-black needles, occupies the entire cell body and finger-like processes of the cells which project over and embrace the outer segments of the visual cells. The nuclei of the epithelial layer are spherical and occupy the choroidal portion of the cell body. Light and darkness have no effect on their shape and position.

Visual cells. The retina of Alligator mississippiensis contains both rods and cones, differing in this respect from the retinae of turtles and lizards (Detwiler, '16). The two kinds of visual cells in the alligator are not uniformly distributed, the cone-rod ratio changing in different parts of the retina. Histological examination of the retina has yielded the significant fact that no portion is rod or cone free and that there is no gradual transition from the conical elements into rod-like forms, as Garten ('07) claims. There are, however, areas which predominate in rods as well as areas which contain only a few rods. Viewing the retina as a whole, it can be said with justice that it is characteristically a rod-retina.

Rods. The structure of the rod is uniform throughout the retina. It consists of an inner segment composed of a cylindrical myoid and an ellipsoid and a cylindrical outer segment (fig. 5). No rods with conical outer segments could be found. The rod nuclei are typically oval in shape and lie just beneath the external limiting membrane projecting above it for variable distances in both dark and light eyes, and thus form the outer part of the external nuclear layer.

Cones. There are two kinds of cones, of which the predominating type is a large thick visual element very similar to that

found in the turtle retina. The inner segment consists of a short broad myoid, a broader refractive paraboloid, and an ellipsoid, while the outer segment is relatively short and conical (fig. 5). This type of cone is found particularly in the posterior and ventral portion of the retina. The second type of single cone (not very numerous), which is found only in the ventral portion of the retina, has a considerably longer myoid and a

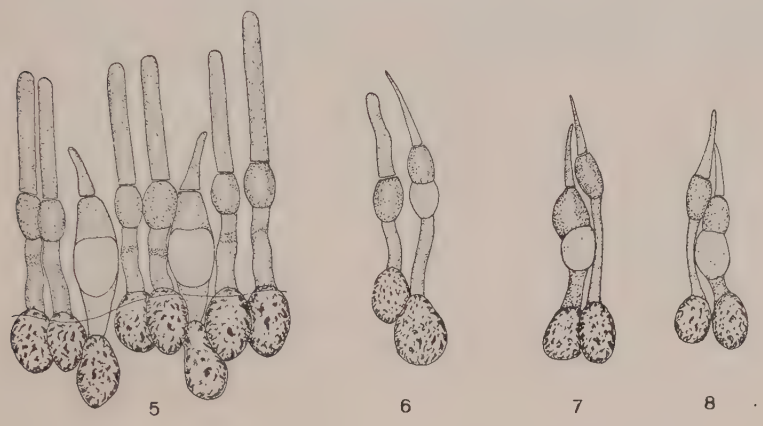

Fig. 5 A portion of the visual layer showing rods and large single cones. × 935. Drawing made at about 2.5 mm. above the entrance of the optic nerve (level C, fig. 1).

Fig. 6 Elongated single cone and a neighboring rod taken from the ventral portion of the retina. × 935. Animal in darkness for twenty-four hours.

Fig. 7 A double cone taken from the lower portion of the retina. Animal in darkness for twenty-four hours. × 935.

Fig. 8 A double cone taken from the tapetal portion of the retina. Animal in diffuse light for seven hours. × 935.

narrower paraboloid. The conical outer segment is long, slender, and pointed (fig. 6).

The double cones consist of a larger and a smaller member. The former are very similar in shape to the large single cones. The latter is characterized by a long slender myoid and the absence of a paraboloid and has not been observed to occur singly (figs. 7 and 8). All cones lack oil drops, differing in this respect from those of the turtle and lizard.

Relative distribution of rods and cones. In the upper peripheral portion of the retina there are very few cones. In the region designated by A (fig. 1), the ratio of rods to cones is about 95:5. The cones in this region, mostly single and of the large type, are irregularly distributed. In the typical tapetal portion (fig. 1, B) they are slightly more numerous (about 15 per cent). The majority of the cones, however, in this region are double, unlike those from the more peripheral portion of the retina. As the optic nerve is approached the number of cones shows a corresponding increase. In the region designated by C (fig. 1)

TABLE I[1]

	DISTANCE FROM NUCLEUS TO OUTER SEGMENT IN μ		DISTANCE FROM NUCLEUS TO ELLIPSOID IN μ		DISTANCE FROM NUCLEUS TO PARABOLOID IN μ			DISTANCE FROM ROD NUCLEUS TO NEAREST PIGMENT NEEDLE IN μ
	Cones	Rods	Cones	Rods	Single cones	Double cones		
						Large	Small	
Dark	19.8	13.5	14.5	7.5	6.8	6.7	13.0	11.5
Light	17.6	17.5	12.1	11.5	4.7	4.0	9.5	9.9
Difference	2.2	4.0	2.4	4.0	2.1	2.7	3.5	1.6

[1] Total number of rod measurements, 280.
Total number of cone measurements, 190.
Total number of pigment measurements, 60.

Measurements made at about 2 to 3 mm. distance from the entrance of the optic nerve, in the region designated by C in figure 1, with the exception of the measurements of double cones which were taken from region B.

approximately 40 per cent of the visual cells are cones. Here the double cones are still more numerous than the single cones. In the guanin-free portion above the optic nerve (fig. 1, D) cones and rods are about equal in number, the single cones again exceeding in number the double. The cones outnumber the rods in the lower portion of the retina, the number increasing from the region of the optic nerve toward the ora serrata, and including all types. In this region only a few scattered rods are present. About 75 per cent of the cones in this region are single, the majority of which are like that shown in figure 5. The small type (fig. 6) is relatively scarce.

The cone nuclei are easily distinguished from the rod nuclei; the former being somewhat pear shaped, the latter more or less oval or elliptical. Furthermore, the cone nuclei occupy a deeper level than the rod nuclei and constitute a second row.

EXPERIMENTAL

Effects of light: Rods and cones. When sections of eyes of animals which have been exposed to diffuse light are compared with sections of eyes of animals kept in darkness, it is seen that there is an average difference in the lengths of the rod myoid of 4μ (table 1). The relative lengths of the rod myoids in the dark and light conditions are shown in figures 9 and 10, as well as

TABLE 2

	DISTANCE FROM ROD NUCLEUS TO ELLIPSOID (MYOID) IN μ	
	Region	
	3 mm. from optic nerve	Tapetum (region B, fig 1)
Light	11.5	8.9
Dark	7.5	6.9
Difference	4.0	2.0

diagrammatically in figure 11. The change in the length of the rods is found to be less extensive in the tapetal area (fig. 1, B), where rods predominate, than in the region close to the optic nerve, where the cones are considerably more numerous (table 2).

The effect of light on the cones is not so easily demonstrable. The results, however, of several series of measurements (table 1) show that the cone myoids of light eyes are slightly shorter (2.1μ) than those of dark eyes (figs. 11, 12, 13). The contraction is found to occur in the double as well as in the single cones. The measurements of the double cones show that the myoid of the smaller member has shortened more than that of the larger (figs. 7 and 8). Further evidence of changes in the length of the visual cells in light and darkness is afforded by a study of the relative positions of the cone and rod ellipsoids. In the dark

condition (fig. 9) the ellipsoids of the single cones are usually found to be on the same level as the rod ellipsoids. On the other hand, in the light condition (fig. 10) the cone myoid is seen to occupy a level closer to the external limiting membrane than that of the rod ellipsoid. This change in relative position is the result of the combined effect of rod elongation and cone contraction,

Fig. 9 A portion of the retina 3 mm. from the entrance of the optic nerve (region C). Animal in darkness for twenty-four hours. × 935.

Fig. 10 A portion of the retina from a region corresponding to that designated in figure 9. Animal in diffuse light for seven hours. × 935.

and is clearly shown by an examination of the rod and cone shown on the right-hand side of the diagrammatic figure 11.

Pigment migration. The differences in position of the pigment in light and dark eyes is slight. A series of measurements of the distance between the rod nuclei and the nearest pigment needle (table 1), as well as measurements of the distance between the

external limiting membrane and the nearest pigment needle, show that the pigment in the light eye is about 1.6μ nearer the external limiting membrane than in the dark eye. This almost insignificant amount, however, when combined with the distance the rod myoid has elongated in the light (4μ) gives an effective migration which is clearly illustrated in figures 9 and 10, as well as in figure 11. An examination of these figures shows that in the dark condition the choroidal portion only of the rod ellipsoid is covered with pigment, while in the light condition the entire

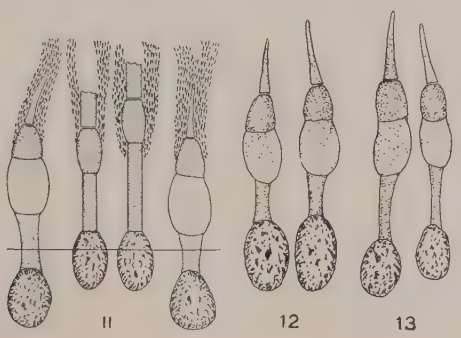

Fig. 11 Diagrammatic drawing compiled from 530 measurements showing the effects of light and darkness on the visual cells and on the position of the pigment. The left-hand side of the figure represents the dark condition; the right side, the light. The drawings were accurately laid out on coördinate paper from measurements presented in table A, each millimeter being given a value of 0.5μ.

Fig. 12 Single cones. Animal in diffuse light for seven hours. × 935.

Fig. 13 Single cones. Animal in darkness for twenty-four hours. × 935.

ellipsoid and a portion of the myoid is ensheathed. The amount of cone contraction in the light, being only slightly more than the extent of the pigment migration (table 1), the relation between the position of the pigment and the cone ellipsoid is practically the same in both dark and light eyes, the choroidal portion of the cone ellipsoid being in both covered by pigment (figs. 9, 10, and 11). The description of the position of the pigment in relation to the ellipsoids of the visual cells in both dark and light eyes pertains only to conditions found in the posterior part of the retina (about 2 to 3 mm. from the entrance of the optic nerve). Changes in the position of the pigment in

the more peripheral region of the retina could not be demonstrated. Near the margin of the retina the visual cells are greatly shortened and the pigment is found to extend down almost to the external limiting membrane.

DISCUSSION

Photomechanical changes of the retina. The question of the functional significance of pigment migration and the changes in position of the visual rods and cones in light and dark adaptation is one about which much has been written (Garten, '07, and Helmholtz, '11). It may therefore appear redundant to add anything in the way of a theoretical consideration of this function. But there are a few points which still lack clarity.

In the eyes of those animals in which these changes take place they represent a mechanism for the adaptation of the eye to day and to twilight vision (Herzog, '05; Exner and Januschke, '06). In dim light (twilight vision), when the rods alone are capable of being stimulated to any degree, or in complete darkness, the pigment moves back and leaves free the spaces between the rods, resulting in a less complete insulation of these elements. Under these conditions, with the entrance of a small amount of light the part played by the individual rods in the reception of the light is greater, owing to refraction and diffusion, than if the rods were covered over by a thick mantle of pigment, in which case only the light which passes through the retina in the direction of the long axis of the rods could enter them. The presence of a reflecting tapetum further enhances the favorable conditions. The cones in twilight vision are not functional, on account of their high threshold, and they elongate and thus move out of the way. The rods contract and thereby optimum conditions are presented for their stimulation.

In bright light (day vision) the pigment by migrating forward protects the rods, which have a low threshold and which have been made particularly sensitive by the accumulation of visual purple in the dark, from too strong stimulation by absorbing the direct and scattered light. The rods elongate, while the less sensitive cones are drawn out of the pigment by the con-

traction of their myoids and are thereby made freely accessible to the stronger light stimulus, thus presenting optimum conditions for their stimulation.

The fact that these photomechanical changes have not been demonstrated in the eyes of man and mammals does not constitute a denial of their taking place in the eyes of other animals, and there is no ground against explaining as above the phenomena in such animals. Adaptation of the mammalian eye is brought about by different means, viz., the formation and bleaching of visual purple.

The process of light and dark adaptation is not dependent upon the phototropic movements of the elements of the retina, but these movements may take part in the process of adaptation, that is in the formation and bleaching of visual purple. That the pigment, as such, has anything to do with the formation of visual purple is not probable, because visual purple occurs in the eyes of albinos and in the pigment-free portions of the retina of many animals, for example, the cat. The significance of the pigment is probably a purely optical one concerned with the absorption of scattered light.

In connection with the function of the retinal epithelium in the formation of visual purple, the paper by Kolmer ('09) is to be noted. Kolmer finds numerous droplets and granules on and between the rods in the retina of various vertebrates. These he regards as secretion products of the pigment epithelium. In the retina of frogs kept in darkness the droplets and granules are larger and more numerous than in the illuminated retina, and after illumination of the eye with direct sunlight are not to be seen at all. Since they are lacking in the eye of lizards and snakes, Kolmer assumes that they have something to do with the visual functions of the rods, the organs of twilight vision, and perhaps with the appearance of visual purple.

It is interesting to note that pigment migration is still assumed by some to take place in the human eye. Ramon y Cajal ('11, p. 363) believes that the function of the pigment is to prevent the impression of halo, and that the dazzling sensation which one experiences on going from a dimly lighted place into a bright one

is due to the fact that the pigment, which has moved back into the body of the epithelial cells as an effect of darkness, requires some time to be brought out again into the prolongations of the cells to ensheath the individual visual elements.

Bard ('19), in a highly theoretical paper, in many respects offering views widely divergent from those usually held concerning vision of form and of color, explains many things by the assumption that both pigment migration and cone contraction take place in the human eye.

Cobb ('19, p. 444) also states that pigment migration takes place in the human retina. He says:

> aside from the changes in the size of the pupil there are two anatomical factors undoubtedly concerned in dark and bright adaptation: the exhaustion and regeneration of visual purple (or possibly other photochemical substance); and the migration of the pigment of the hexagonal cells. This last may be a protecting device that acts fairly promptly, and has the effect of enclosing the retinal rods, and by its own light-absorbing qualities reducing the amount of light absorbed by the individual rods. It is conceivable that a sudden flash of light might anticipate this action and produce a strong destruction of the photochemical material in a short time, before the pigment cells have had time to react, while with gradual onset of light the time is adequate for the pigment cells effectively to assume this protective function.

And on page 445:

> Some of the curves strongly suggest two factors playing a part in dark adaptation . . . . allowing the interpretation that the results are arising from two more or less independent mechanisms one of which overtakes the other in effect, at the end of about four minutes.

We believe that in the migration of pigment, the contraction of the cones, and the elongation of the rods there is exemplified the response of irritable protoplasm to a definite, adequate stimulus. In some cases the response is very marked, though of varying degree (fish, frog, bird); in others it is not demonstrable (man and mammals). In some it may serve an easily comprehended purpose; in others, in terms of the theory explaining it, it may seem to be useless. Nevertheless, if it can be demonstrated (as in the turtle, lizard, and alligator) it cannot be explained away or ignored because it seems to serve no useful purpose.

In the eye of the alligator the migration of the pigment and the change in position of the visual cells seem to be correlated with the relative distribution of rods and cones. The rods show the greatest difference in position in light and dark eyes, in the regions designated by C in figure 1 (rod-cone ratio 60 to 40), and by D (where the rods and cones are about equal in number), much less in the region B (rod-cone ratio 85 to 15), and not demonstrably at all in the region designated by A, or in the region (E and F) below the optic nerve, where the rods represent only about 5 per cent of the total number of visual cells. The pigment can be demonstrated to move forward only in the posterior portion of the retina (regions C and D), thus corresponding to the regions where the maximum change in position of the rods takes place. The cones throughout the retina show the same (slight) degree of shortening in the light, except that the double cones, which are most numerous in the regions B and C, show a slightly greater amount of change in length.

Garten ('07, p. 38) weakened the general application of the suggestion put forth by Herzog ('05) and Exner and Januschke ('06) by observations which seemed to show an extremely high sensitivity to stimulation by weak light, so that the light condition of the visual cells was considered as assumed in dim light. Arey ('19) has recently brought forward evidence indicating that the sensitivity of the retinal pigment and of the rods and cones is nowhere nearly so high as is generally believed, and the conception that the changes observed in those eyes where marked effects of light are obtained are adaptive has been thereby placed on much surer ground.

We should not, however, a priori, deny that light effects similar, if less marked, changes in retinae so constituted that there can be no, or little, question of any advantage to be gained by a correlative shifting of the position of the visual elements. Garten cites the facts that in the selachians, which presumably have pure-rod retinae, although the literature on the subject is not without disagreement,[1] there is little, if any, pigment, and that

[1] Schultze, '66; Krause, '76 and '95; Neumayer, '97; Schaper, '99; Hesse, '04; Franz, '05; Retzius, '05; Garten, '07; Cajal, '11, and Pütter, '12.

in pure-cone retinae, where the pigment, as assumed, is necessary for the absorption of the light scattered by the highly refractive cones, there is practically no pigment migration. But Detwiler ('16) found a demonstrable pigment migration and cone contraction in the eyes of both turtles and lizards. Garten further argues (p. 109) that photomechanical changes should not take place in the crocodilian eye, because of the exclusive presence of rods or cones in the various portions of the retina. But, as we have demonstrated above, the structural conditions, at least in Alligator mississippiensis, differ from his description, and changes in the position of pigment and of visual cells do take place in light and darkness.

The duplicity theory. The duplicity theory of von Kries, or the theory of the double retina of Parinaud, based on the findings of Max Schultze ('66), is of the greatest importance in comparative work on vision. The hypothesis is generally regarded as well substantiated, particularly by the facts of twilight and day vision. For brief accounts and references to the literature of the theory and its development the reader is referred to Nagel ('05), Helmholtz ('11), and Parsons ('15). Briefly stated, the theory holds that the rods are sensitive only to light and darkness, and by virtue of their power of adaptation in the dark through the regeneration of visual purple they form the apparatus for vision in dim light. The cones, on the other hand, are the apparatus subserving bright vision as well as the perception of color. But in another way, the rods are the apparatus for achromatic scotopic vision (twilight vision), the cones the apparatus for photopic vision (day vision). The cones are not necessarily assumed to be utterly useless at night, but only relatively so, being quickly fatigued, on account of their high threshold.

The presence and relative distribution of rods and cones is therefore a matter of the first importance. But without prejudice it can be said that this is a very unsatisfactory matter as far as the comparative literature is concerned. Early contributions to the histology of the visual neuro-epithelium either have not been reinvestigated, but assumed to be correct, or

when investigated a divergence in the opinions of later investigators is the rule rather than the exception.

The question as to what constitutes a rod and what a cone would seem to be a simple matter, but the great variety in the forms of the cells in different animals makes difficult a generalized classification. The contentions of Pütter ('12) for a functional classification based on the type of connection of the visual cell with the bipolar, rather than a structural one, are good if kept within limits, but it seems to us that they are carried too far. As everyone knows, the foveal cones of man and mammals are cylindrical in shape, and are therefore much more like rods in general appearance than cones. But their known function fits in with the general conception of the physiology of the apparatus for color and bright-light vision. Troland ('17) has pointed out that the shape of the foveal cones suggests that the function of the cone figure is structural rigidity rather than differentiation of response.

Whether a visual cell is a rod or a cone is determined by the presence of one or more of three structural factors, viz., 1) the shape of the outer segment; 2) the shape of the inner segment, and, 3) the mode of connection between the visual cell and the bipolar cell. When we find visual cells which, from their general form (outer and inner segments), would be called rods, possessing terminal connections typical of cones (e.g., frogs, diurnal birds, see Ramon y Cajal, '94, pp. 31, 164, and '11, pp. 340, 327), there is no, or very little, reason why they should be called cones simply because they terminate in dendrites, and considerable reason for continuing to designate them as rods on functional as well as structrual grounds. The conditions in the geckos may be cited as another example. From description and illustration it would seem as if no more typical rods could be found. Coupled with their structure there are functional characteristics, which will be referred to later, and which, from all that we know about rod vision, indicate that the visual cells are as functionally typical rods as can be found. Our work on the retina of the alligator shows that rods as well as cones occur there, structurally as well as functionally, as exemplified in the inverse changes in

light and darkness. But Pütter would call them all cones because their centripetal termination is similar to that found in the cones of man.

The designating a visual cell as a rod or as a cone on morphological grounds is not therefore useless, as Pütter claims, but, as he also points out the structural basis (form of inner and outer segments), is brought into line with the functional by what we know of the respective functions of the visual cells in man, viz., threshold, visual acuity, ability to see movement, and vision of color. The rods are visual elements with a low threshold, but with possibilities of summated conduction, due to the connection of more than one of them with a single bipolar cell; the cones are visual elements with a high threshold and isolated conduction, based on the histologically found type of connection.

Pütter, in speaking of the conditions in the nocturnal birds, admits that the visual elements have knob-like endings, and that the visual cells are morphologically typical cones, although they have assumed what he regards as the most distinctive characteristic of rods. Pütter reverses himself here and is, as well, incomplete, because, as Ramon y Cajal ('94, p. 104) points out, in these retinae there are rods ending like those of mammals, while the cones which have almost entirely similar endings, reach deeper and come into connection with a different set of bipolars, so that there is a further morphological differentiation here between rods and cones.

It does not seem at all certain to us that Hess ('10 and '13), by his work on the turtle and on birds, has disproved or weakened the general truth of the duplicity theory. He claims to have demonstrated an adaptation in the turtle retina, where there are cones only. The claim that he and Katz and Révész (13) make, that adaptation in diurnal birds is a function of the cones, does not seem warranted, owing to the fact that rods are present in considerable numbers, as Hess himself describes, particularly in connection with the presence of visual purple. The phenomenon, similar to the Purkinje phenomenon, which they state to have observed, may therefore, and most naturally, be a function of the rods and not of the cones. Katz and Révész

('13) also state that the rods of nocturnal birds (owls) in bright light approach, or are similar, in function to cones. But this is without anatomical foundation for the simple reason that the retinae of such birds contain numerous cones (Garten, '07; Hess, '13, p. 581) which show, with the pigment, photomechanical changes. In this connection the view of Parsons ('15, p. 204) may be quoted:

> If we regard the rods as the more primitive type of visual neuro-epithelium, as we are probably justified in doing, the persistence of recognizable rod attributes in the cones, even if modified, differentiated, and rendered more complex, might well be expected. Apart therefore from the difficulties of isolating the physiological results of excitation of the rods from those of excitation of the cones it may be anticipated that the latter cells will retain some measure of the functions which are in the highest degree characteristic of their prototypes. Hence, if it should ever be conclusively proved that the rod-like foveal cones of the human eye possess some trace of visual purple and are endowed with some slight degree of light-adaptation it would not be surprising; neither, on the other hand, would it militate seriously against the view that the rods and cones have become essentially diverse in function.

Troland ('16) has demonstrated by careful experiments, corroborating the earlier work of v. Kries and Nagel, that the phenomenon of Purkinje does not take place in the rod-free portion of the human retina. And Watson ('15) and Lashley ('16) show that Hess' contention that the spectrum is shortened for the bird's eye as compared with the range of wave lengths seen by the human eye, is not correct.

It is not out of place to add that in the condition known as night-blindness, in which the rods are insensitive, or practically so, dark adaptation is almost abolished or is much slower than normal, and that Purkinje's phenomenon is much less marked than in the normal eye or absent altogether.

One further remark concerning Hess' work. He ('10) claims that many turtles are nocturnal and cites authorities supporting his contention. Ramon y Cajal ('11, p. 361) says that reptiles in general (in which of course he is incorrect, witness the alligator and the gecko) do not see in darkness. Rochon-Duvigneaud ('17) does not believe that turtles can be called nocturnal because they are incapable of flight from an enemy or of pursuing

prey, and he thinks that they detect the plants and insects upon which they feed by the sense of smell. Rochon-Duvigneaud asks the very apt question in reference to Hess' claim of adaptation in the fowl, why it is, if they possess the power of adaptation almost as well-developed as that of man, that they go to roost long before man ceases to enjoy good vision.

With reference to the duplicity theory and the distribution of rods and cones, the work of Abney ('16 and '17) is most interesting and important. By determining the minimum intensity of light of various wave length which can be perceived at the fovea and up to ten degrees from its center Abney and Watson ('16) obtained results indicating that in some cases the fovea of man is free from rods, which increase rapidly as the fovea is left, while in others there is a plentiful supply of rods at the fovea, their distribution, at any rate up to ten degrees, being very nearly uniform, and, if anything, in excess at the fovea. In the first group the light appears colored as long as it is visible at all, particularly in the green. In the second group the light loses color a considerable time before it is extinguished, except in the red. In other words, there is an achromatic interval. Abney ('17) later examined persons suffering from night-blindness, in which the rods are generally believed to be non-functional, for extinction of color from the red to the blue. The light was found to vanish when its color was extinguished, so that the same reduction in intensity of the light was the threshold for both light and color, similarly to the cases mentioned above where there are no rods at the fovea, indicating that there is an absence of sensitive rods in the whole retina of the night-blind.

If dark adaptation is directly associated with visual purple the vision of an animal possessing rods only as compared with that of an animal with cones only, both in respect to ability to see in light and darkness, after longer or shorter adaptation to the one or the other condition, and as well the relative stimulating value of spectral lights of equal energy, should be expected to be markedly different, quantitatively as well as qualitatively. Now we have in the reptiles admirable subjects for investigating this

very question, especially in the lizards, for example, the geckos as compared with other lizards and particularly the horned toad which has a retina, from all structural indications, peculiarly adapted for day vision only. By investigating in the gecko and the horned toad the relative visibility of wave lengths of equal energy and the relative powers of adaptation, we will obtain information concerning the question of the selective response of rods to different wave lengths as compared with that of cones. Visual purple absorbs all wave lengths except a little red and violet. The rods therefore are presumably sensitive to all wave lengths except the extreme red and violet. Since rods are in general 'color-blind,' there is opportunity here of differentiating between wave length and stimulating value.

In connection with the question as to which kind of visual cell represents the more primitive condition, the histogenesis of the retina is worthy of investigation. But the histogenesis of the neuro-epithelium is a subject about which our knowledge is most imperfect. In general, the rods are regarded as the more primitive type of visual cell (Graham Kerr, '19), while the cones are considered as specialized rods.

According to Leboucq ('09), the two kinds of visual elements develop simultaneously and are distinguishable only by the fact that the axis of the diplosome is perpendicular to the surface in the case of the rod and parallel to it in the case of the cone.

Cajal ('11, p. 356) says that the cones and rods evolve in the same way and that it is difficult to distinguish between them at the beginning (also Fürst, '04).

Cameron ('11) reiterates a view earlier championed by himself ('05) as well as by Bernard ('03) to the effect that the cones represent early stages in the formation of rods. We mention this here because of the fact that in looking over some slides of early amphibian embryos, the eyes do seem to contain nothing but cones or conically shaped elements. Graham Kerr (p. 137) finds that the visual elements (rods) in the retina of Lepidosiren shorten in the light and elongate in the dark, which is similar to to the usual behavior of cones and contrary to that of rods.

The shape of the pupil is a subject of interest in a paper dealing primarily with the eye of the alligator because of the vertical slit form of the pupil in this animal. In two recent articles reference is made to this subject. Rochon-Duvigneaud ('17), in listing the characteristics of the eye of the geckos which make them adapted to nocturnal vision, includes the form of the pupil—a vertical slit—which shows a rapidity of movement surpassing that of the human pupil and approaching that of birds. In dim light the pupil is a large oval, or even round, as in the cat. In bright light it is closed completely. According to Rochon-Duvigneaud, a round pupil can dilate as well as an oval one, but it cannot be entirely closed, and he believes that it is in the way of a protection against an excess of light in an animal adapted to twilight vision that an oval pupil finds its chief function. It is possible to imagine that a pupil in the form of a vertical slit can be opened wider than if it were round (for example, the cat).

Hartridge ('19) views the function of a vertical slit pupil (as seen in the cat) from another angle, viz., the function of the lens and the aberrations caused thereby, and the habits of life of the cat family in the nature of their being tree-climbing and tree-dwelling animals which hunt their prey chiefly at night. An oval pupil in which the long axis is vertical will cause the lens system to form images in which the aberrations of horizontal contours are greater than those belonging to vertical contours. The contours of trees and their branches are principally vertical, therefore if the illumination of the image formed on the retina could be increased by sacrificing the definition of horizontal contours it would be an advantage. This is effected by the use of the oval pupil since the aberration of vertical contours is little greater than that of a circular pupil of the same horizontal diameter, while the intensity of the image formed on the retina is as much the greater as the vertical diameter of the oval is greater than that of the circular pupil.

It seems more likely to us that the function of a vertical slit-shaped pupil is for protection; that is, to permit of its being almost, if not entirely, closed. In thinking of animals that have

this type of pupil, we find that many of them are essentially nocturnal in habits—the gecko, the alligator, the cat—animals in which the rod functions are predominant over those of the cones. At night or in dim light the pupil of the cat is wide open and round. Furthermore, in the daytime, when the pupil is a vertical slit or oval, cats hunt and catch a great deal on the ground, for example, birds and squirrels, as well as chase the rapidly swirling leaves. The cat is furthermore said to have very defective daylight vision and to be colorblind (DeVoss and Ganson, '15). The alligator hunts along horizontal contours, and yet one finds that the shape of the pupil is a vertical slit.

SUMMARY

1. The eye of the alligator possesses a well-developed retinal tapetum in the dorsal and posterior portions of the retina to within 1.5 mm. of the entrance of the optic nerve. It is formed by the inclusion of guanin in the cells of the epithelial layer (figs. 1, 2, 3, 4). The pecten consists of a slightly raised pigmented cap covering the entrance of the optic nerve.

2. Typical rods and cones occur throughout the retina, the cone-rod ratio being different for different regions, but characteristic for particular regions (p. 216).

3. The rods are all of one type (fig. 5), the cones of two, thick and thin, of which the first is by far the more numerous, occurring particularly in the posterior and ventral portions of the retina. Those of the second type are found only in the ventral portion and are not numerous (fig. 6). Double cones also occur (figs. 7 and 8). None of the cones contain oil drops. The rod nuclei are oval or elliptical in shape, and the majority of them project through the external limiting membrane for a variable extent, the rest of them being just under it. The cone nuclei are pear shaped and, occupying a deeper level than the rod nuclei, constitute a second row (figs. 5, 6, 9, 10).

4. The rods show a change in length of their myoids averaging 4μ, being longer in the light and shorter in the dark (figs. 9, 10, 11 and table 1). The single cones (thick and thin) show an average change of 2.1μ (figs. 11, 12, 13 and table 1). The

smaller member of the double cones shows an average change in length of 3.5μ, the larger member of 2.7μ (figs. 7, 8 and table 1).

5. The actual change in position of the pigment between light and dark eyes is slight, averaging but 1.6μ; but when combined with the change in length of the visual cells, gives an effective migration equal to the sum of the two (figs. 9, 10, 11).

6. A theoretical consideration of photomechanical changes and of the duplicity theory from a comparative point of view is appended.

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Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1921, xviii, pp. 137–138.

64 (1646)

Paramecium calkinsi sp. n.

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There are at present four well-established species of *Paramecium* (*P. aurelia*, *P. caudatum*, *P. bursaria*, and *P. putrinum*) which fall naturally into two quite clearly defined groups. One group comprises *Paramecium aurelia* and *Paramecium caudatum* which are characterized by a relatively long spindle-shaped body. The other group includes *Paramecium bursaria* and *Paramecium putrinum* which exhibit a somewhat shorter and broader form, with a tendency toward a dorso-ventral flattening. All the species have a single micronucleus except *Paramecium aurelia*, which has two micronuclei each showing characteristic 'endosome' structure.[1]

The purpose of the present paper is to record the discovery in January, 1920, of a hitherto undescribed form of *Paramecium*. It has now been extensively studied in pedigree cultures for more than a year and during this time it has bred true. I therefore definitely designate it a new species, *Paramecium calkinsi*, in recognition of the fact that Professor G. N. Calkins of Columbia University introduced students of the Infusoria to exact, daily isolation, pedigree culture methods.

The general body form of the new species places it at once in the bursaria-putrinum group, but its micronuclei both in structure and number are identical with those of *Paramecium aurelia*. In brief, *Paramecium calkinsi* represents the '*aurelia*' type of micronuclear complex in the '*bursaria*' group of species.

Details of the structure and life history of *Paramecium calkinsi* will appear in the *Biological Bulletin*.

[1] H. S. Jennings and G. T. Hargitt: "Characteristics of the Diverse Races of Paramecium," *Journal of Morphology*, 1910, xxi, 495. L. L. Woodruff: "*Paramecium aurelia* and *Paramecium caudatum*," *Journal of Morphology*, 1911, xxii, 223.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 28

Reprinted from THE AMERICAN JOURNAL OF ANATOMY, Vol. 28, No. 3, March, 1921

A STUDY OF THE DEVELOPMENT OF CONNECTIVE TISSUE IN THE AMPHIBIA

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SIX FIGURES (FOUR PLATES)

INTRODUCTION

The studies in which the author has been engaged for several years have shown, in brief, that the plasma clot obtained from frog's blood is of such a nature that, when influenced by the proper factors, it is possible to radically transform its structure. The reactions of the clot were studied in tissue cultures ('15) and in wound healing ('16). In both these cases it was possible to demonstrate clearly that the clot could be transformed into a fibrous tissue which was apparently identical in structure, save for the absence of connective tissue cells, with regular connective tissue. In wounds made in frog skin, the experiments further showed that the fibrous tissue which developed from the fibrin clot functioned, at least temporarily, as a normal connective tissue and there was no evidence to indicate that it would ever be replaced. In the final paper of the series ('17)—in which a study of the normal clotting and the transformation of the clot under the influence of certain mechanical factors was studied with the aid of a microscope equipped for dark field illumination—it was possible to demonstrate that the transformation of the clot was brought about by a fusion and consolidation of the fine elements of which it is composed. This process resulted in the formation of long fibers which united to form wavy, fibrous bundles. These bundles of fibers anastomosed with other bundles, ramified in various directions throughout the clot and the result was the formation of a fibrous material which closely resembled regular connective tissue.

The above results led naturally to the question as to whether a similar process normally takes place in the histogenesis of the connective tissues in amphibian embryos and thus to the present study in which this problem is considered. It is worthy of note that, notwithstanding the great amount of work that has been done upon the development of connective tissue, no general agreement has been reached. At present there are two main theories held regarding connective tissue formation—the intracellular theory and the intercellular theory—and, in general, the results of the various investigators support one or the other of these. In order to get the matter clearly in mind it may be well to set forth a statement of the opposing views, which are as follows:

According to the intercellular theory there is early formed in development an amorphous, gelatinous, non-living ground substance. It is generally held that this is formed as a secretion of the mesenchyme cells. Later, fibers are formed in this ground substance by a deposit or secretion which is given off by the cells of the tissue. It is held that the cells have no morphological connection whatever with the fibers which form in the ground substance.

The intercellular theory substantially as stated above was the original theory held for connective tissue formation and was first stated by Henle. With various minor modifications, it has been supported by v. Kölliker, Merkel, Ranvier, v. Ebner, Renaut, Schäfer, Laguesse, and others. The material used in the researches of these investigators was obtained from many different sources and included both embryonic and adult tissue.

The intercellular theory has been, perhaps, most strongly supported by Merkel. In his last paper ('08) he gives a splendid review of the question of connective tissue development as well as the results of his own studies. His conclusions are that the primitive origin of all connective tissues lies in an amorphous gelatinous substance which is secreted by the syncytium of the mesenchyme cells. This secretion constitutes the ground substance and in some cases it is formed sparingly and in other cases very abundantly (e.g., Amphibia, umbilical cord). The fibers,

according to Merkel, are really strands of the amorphous ground substance and they increase in size through an assimilation of the surrounding ground substance. The cells play no direct part in the formation of the fibers but serve only to produce the ground substance by secretion. Merkel also believes that the formation of the connective fibers is due in every case to the influence of mechanical factors acting on the ground substance; e.g., where there is a decided stretching, as in a tendon, parallel fibers are formed from the ground substance.

According to the intracellular theory, the ground substance which is early formed is regarded as living material, although it is intercellular in position. The fibers which form in this ground substance are considered by some investigators to be strands of cell cytoplasm which have been given off from the periphery of the connective tissue cells. Others believe that a transformation of the entire cell into fibers takes place. In either case, in the first stages of fiber development, all the fibers are regarded as being merely modified strands of cell cytoplasm. The fibers when once formed by the cells may, and often do, become separated from the cells but nevertheless it is held that they are able to grow both in thickness and in length by intussusception due to the assimilation of food material. The living intercellular material is also able to grow in the same manner and it increases greatly in amount in proportion to the cells present.

The intracellular view has been modified by the Hansen-Mall theory, which is widely held at present. In accordance with this theory, the ground substance arises as a result of a syncytium of the mesenchyme cells and is therefore regarded as being a common living exoplasm of the mesenchyme cells. In this exoplasm, each of the nuclei of the mesenchyme cells are surrounded by a minute mass of cytoplasm which constitutes the endoplasm of the cells. According to this theory the fibers arise in the common exoplasm either in the regions in immediate contact with the cells or in the regions of the exoplasm which may be far removed from cells.

The above view regarding connective tissue formation is clearly set forth by Mall ('02) who says:[1]

> In very early embryos the mesenchyme is composed of individual cells which increase rapidly in protoplasm and then unite to form a dense syncytium. The protoplasm of the syncytium grows more rapidly than the nuclei divide, so that in a short time we have an extensive syncytium with a relatively small number of nuclei. In its form the syncytium appears as large bands of protoplasm with spaces between them filled at times with cells and at other times with fluid. The second condition we have in the umbilical cord of young human embryos. About this time the protoplasm of the syncytium differentiates into a fibrillar part, which forms the main portion of the syncytium—the exoplasm—and a granular part, which surrounds the nucleus—the endoplasm.

The view of Mall, as stated above, is in quite close agreement with that of Hansen ('99 and '05) which is based upon his work on the development of cartilage. He believes that,[2] in many cases, it is not possible to distinguish between protoplasm and ground substance and also that[3] cells of cartilage are to be considered as an endoplasm and the ground substance as a common ectoplasm which is more or less independent of the endoplasm.

In the concluding section of this paper will be found an analysis of these two theories and a discussion of the relations shown to them by the results obtained from the present work. It may, however, be stated at this time that the results reported in the present paper[4] strongly support the intercellular theory of connective tissue development, and furthermore they show that the process of connective tissue formation closely resembles, morphologically at least, the formation of fibrous tissue by transformation of a plasma clot.

[1] Mall, 1902, p. 331.

[2] Hansen, 1899, p. 434.

[3] Hansen, 1905, p. 747.

[4] A report of this work was given at the meeting of the National Academy of Sciences held in New Haven, Nov., 1919, and an abstract of the work is published in the Proceedings of the National Academy, 1920, vi, 77.

MATERIAL AND METHODS

In the present work, embryonic amphibian material has been used. It was obtained from a number of species, chiefly Rana sylvatica, R. palustris, R. catesbiana and Amblystoma punctatum. From each of these species, five series of nine or ten embryos, each ranging from the medullary plate stage by regular gradations to well developed free swimming larvae were obtained for preservation. In all about 200 embryos were selected for permanent preservation. In addition to this, through the kindness of Professor R. G. Harrison, access was had to several series of transverse, sagittal and frontal serial sections of R. palustris, cut 7.5μ and stained in haematoxylin and Congo red. Figures 1 and 2 are taken from this series.

The following preserving fluids were used: *a*) Zenker with 5 per cent glacial acetic acid; *b*) Zenker without acetic acid; *c*) saturated solution of mercuric chloride with 5 per cent glacial acetic acid; *d*) saturated solution of mercuric chloride without acetic acid; *e*) 80 per cent alcohol. Several series of embryos were preserved in these fixing fluids. They were later imbedded in paraffin, and transverse, sagittal and frontal serial sections were made ranging from 7 to 10 micra in thickness.

The following stains have been used: *a*) Delafield's haematoxylin; *b*) iron haematoxylin; *c*) picro-fuchsin connective tissue stain; *d*) Mallory's anilin blue connective tissue stain, and *e*) Mall's modification of the Mallory stain. With the large amount of material that was available, preserved by various methods, and with the various methods of staining which were used, it was possible to test a large number of combinations and to determine the combination which was best adapted for the present work. Considerable time was spent in making this comparative study of the preserving fluids and stains and their reactions with the tissues, and it is believed that the combination, consisting of Zenker with acetic for the killing fluid and Mallory's aniline blue connective tissue stain which was finally adopted and used for most of the material is the best possible one for the problem in hand.

With regard to this combination, it may be well to say in the first place that, of all the preserving fluids tried, Zenker with acetic gave the best results. The only objection to this fluid is that it has a tendency to harden the yolk material in the embryos somewhat more than some of the other fluids and this makes the sectioning of the earlier stages difficult. However, the difference here is not great and the superior preservation in the Zenker fluid warrants its use.

As to the staining, it was found that the Mallory connective tissue stain was superior to any of the other stains tried for the work in hand. A comparative study was made of the reactions of this stain as originally given and the same stain as modified by Mall ('01–'02). The results obtained show very clearly that the modified Mallory does not give such a clear differentiation between cytoplasm and ground substance as does the original Mallory stain. Mall says,[5] "My best specimens were obtained by staining the sections with Mallory's connective tissue stain which tinges the nuclei and surrounding endoplasm, if present, slightly red and the exoplasm of the syncytium intensely blue. We have modified this stain somewhat by omitting the water and int nsifying the blue."

The results of the comparative study have convinced me that the intensification of the blue is a mistake in that thereby the differentiation between the cell cytoplasm and the ground substance of the developing connective tissue is largely lost. In material preserved in Zenker and correctly stained with Mallory's original stain there is a clear differentiation between the cells, which are of varying shades of red, and the ground substance which is invariably blue. It is possible therefore in such material to speak with certainty as to the limits of the cell boundaries and the relations the cells bear to the common intercellular ground substance. When the blue is intensified the entire field takes this color and the differentiation becomes obscured or even lost.

[5] Mall, 1902, p. 338.

STUDY OF PREPARED MATERIAL

If sections of a frog embryo, taken shortly after the notochord is formed and before the tail bud stage is reached, are studied, it will be found that the notochord of the embryo is surrounded by a mass of transparent gelatinous material (primitive ground substance). Such a stage is shown in figure 1 which was made from a portion of a frontal section of a 3.2 mm. embryo of Rana palustris. The magnification is 285 diameters. Above a portion of the central nervous system is shown. Extending posteriorly, in the median line, is the notochord imbedded in a gelatinous cell-free matrix or primitive ground substance which extends laterally between the muscle plates in either direction.

In figure 2 is shown a portion of a transverse section through a 4 mm. embryo of the same species but slightly older than that shown in figure 1. The magnification is 322 diameters. The notochord is shown in the center of the figure. Lying above it is a portion of the ventral wall of the medullary tube. The embryonic muscle cells can be seen lying to the right and left of the notochord. Filling the space between the notochord and the muscle cells is the gelatinous material mentioned above, which constitutes the primitive ground substance. This material also extends dorsally along the sides of the spinal cord and laterally into the muscle tissues. As is shown in the figure, the ground substance in some regions shows a fine fibrillation running through it. In other regions it appears homogeneous.

At the stage in embryonic development shown in figures 1 and 2, the mesenchyme cells, which later wander all through the cavities of the embryo, are not present in this region in which the primitive ground substance is first formed. The case is clear, therefore, that the ground substance which is here present surrounding the notochord has not arisen by syncytial fusion of cell cytoplasm.

The primitive ground substance having been formed as shown in figures 1 and 2, it is next found that, in an embryo of a little later stage, cells begin to detach themselves from the cell masses and wander through it. These cells, when they first appear,

are for the most part more or less rounded in shape and contain a considerable amount of yolk. As they move through the ground substance they become amoeboid and also in some cases spindle-shaped; a form which is regarded as being typical of the so-called connective tissue fibroblasts. It is frequently possible to trace the movements of the cells by the altered appearance of the gelatinous ground substance.

In figure 3 is shown a portion of an unstained transverse section through a 9 mm. embryo at a magnification of 322 diameters, at the stage when the cells are starting to move into and through the ground substance, as described just above. In this figure a portion of the notochord is shown with embryonic muscle tissue lying on either side. Both the notochord and the muscle tissue are imbedded in a matrix of the ground substance which is especially heavy in the region just below the notochord. From this region, it spreads to the right and the left surrounding the bundles of muscle fibers and extending to the body wall on either side. At the stage shown numerous cells are to be seen in the portion of the ground substance which lies below the notochord. These cells vary considerably in shape; some are rounded, while others have assumed a spindle-shape.

In figure 4 is shown a portion of a transverse section through the tail region of an 11 mm. embryo which was preserved in Zenker and stained with Mallory's stain. The magnification is 760 diameters. The portion of the section shown in this figure lies just below the notochord. At this point there is a considerable area of the primitive ground substance. It is surrounded ventrally and laterally by developing muscle tissue and dorsally by the notochord. In this figure it is desired to lay particular emphasis upon the relations shown between the ground substance and the cells which are present in it. The cells which have wandered into this region have for the most part become amoeboid or spindle-shaped in type and they possess long cytoplasmic processes which stretch out in various directions through the ground substance. The figure shows that, in a preparation of this kind which has been properly stained with the Mallory connective tissue stain, the differential color reaction

between ground substance and cell bodies is so clear that the cell processes can be differentiated from the ground substance and traced to their definite endings, even though they may be drawn out extremely fine. Attention should also be called to the color reactions of the muscle tissue which bounds the ground substance. It will be noted that the bundles or muscle fibers show the characteristic color reaction of the cytoplasm of the mesenchyme cells lying in the ground substance. These results demonstrate that the Mallory connective tissue stain differentiates definitely the living substance, present in the cells and in the muscle tissue, from the intercellular ground substance.

It is worth while to note in this connection that Mall says[6] "In specimens of this kind it is easy to view these cells with their endoplasm as the connective-tissue cells and the exoplasm of the syncytium as the intercellular substance were not the development of the syncytium taken into consideration." And also[7] "while my results are now decidedly in favor of Flemming's view, the reader will soon see that if other methods and interpretations are employed (which I now consider false), it will be quite as easy to see the fibers developing between the cells as within them." Inasmuch as in the present work the study of the various stages has shown, in general, that the formation of the ground substance takes place before there is any attempt to form a syncytium by the mesenchyme cells—in fact before they have left the cell groups, wandered into the ground substance and there assumed the irregular shapes characteristic of mesenchyme cells—it is evident that the formation of the ground substance cannot be due to a cytoplasmic syncytium. This fact leads definitely to the conclusion, as Mall suggests above, that the cells with their endoplasm are the connective tissue cells complete and the material in which they lie is not exoplasm but an intercellular substance or ground substance. The cells which move into the common, intercellular ground substance are separate entities; not a part of it but apart from it.

[6] Mall, 1902, p. 336.
[7] Mall, 1902, p. 330.

The presence of a ground substance formed prior to the appearance of the mesenchyme cells has been demonstrated by various investigators but notably by Szily ('07) who, working on chick and fish material, says[8] "Vor dem Auftreten der Mesenchymzellen sind die Lücken and Spalten der Embryonalanlage durch ein feines Fasersystem ausgefüllt." He holds, however, that this cell-free, fibrous, supporting tissue, instead of being a secretion from the cells, arises from fine fibrous protoplasmic processes of the epithelial and endothelial cells. The mesenchyme cells appear later and enter into a protoplasmic union with the fibers already present. The embryonic connective tissue is made up of the mesenchyme cells and the fibrillar intercellular material. The mesenchyme cells furthermore serve for the nourishment and growth of the fibers which, in the meantime, have become separated from the cells of which they previously formed a part. Szily therefore believes that, in the formation of the embryonic supporting tissues, all three of the primary germ layers take an active part, and the final product is a mixed tissue in which the product of any one germ layer cannot be identified.

The present work agrees with the work of Szily in demonstrating the presence of a primitive ground substance prior to the advent of the mesenchyme cells. No evidence has been found, however, of its formation by means of protoplasmic processes of the surrounding cells. The study of the embryonic cells in the amphibian material, at the early stage at which the primitive ground substance is formed, shows them to have a clear, sharply defined, regular membrane with no processes of any kind. There is no question but that with a differential stain, such as Mallory's which was used, protoplasmic processes could be demonstrated if they were present. It is easy enough to do so a little later with the mesenchyme cells when they wander into the primitive ground substance as shown in figures 4 and 6. The conclusion is, therefore, that the primitive ground substance in the amphibian embryo arises as a cellular secretion and not by the fusion of cytoplasmic processes.

[8] Szily, 1907, p. 741. Cf. also Isaacs, 1919.

In the discussion of the earlier stages, it has already been noted that not only is the ground substance formed prior to the advent of the mesenchyme cells but also, in some regions, it shows from the earliest stages a fine fibrillation. In the stage shown in figure 4, this fibrillation of the ground substance has markedly increased and it is found that practically all the ground substance is permeated with the fibers. In some regions the fine fibers have united to form fibrous bundles and in general there appears to be a tendency for this to take place. A careful study of this and similar preparations reveals the fact, as is depicted in the figure, that there is no morphological connection between the cells which are present in the ground substance and the fibers. The latter have formed directly from the ground substance by a modification of the elements of which it is composed.

The preparations give evidence that the movements of the cells through the ground substance may supply, in part at least, a mechanical factor which aids in the formation of the fibers. For example, it will be noted in figure 4 that the fiber formation is particularly heavy in the portion of the ground substance lying near the ventral opening in the muscle tissue through which, apparently, a number of the amoeboid cells have wandered into the ground substance. The preparations show many similar instances of regions in which the movements of the cells through the ground substance have supplied a mechanical stimulus and thereby brought about the fiber formation. There is also the possibility that the cells in their movements may have some secretory action which also modifies the ground substance. However, this point is clear from the study of the preparations and should be emphasized that in no case has any morphological connection been found between the cells in the ground substance and the fibers.

In this connection attention should be called to the results previously obtained by the author ('17) in certain of the studies on the plasma clot in which it was demonstrated that, under the influence of certain mechanical factors, it was possible to transform a homogeneous fibrin clot into a fibrous tissue which, in its morphology, very closely resembled various types of fibrous

connective tissues. In such cases it was possible to observe all stages in the process from an apparently homogeneous ground substance or matrix to the heavy fibrous tissue. By the use of a microscope equipped for dark field illumination it was possible to analyze the transformation process and to demonstrate that the fibers arose as a result of a fusion and consolidation of the minute elements of the fibrin clot which under ordinary illumination were entirely invisible. In experiments of this type, inasmuch as cells were not present, it was possible to state with absolute certainty that the fibers had arisen through a modification of the ground substance of the clot.

In other types of experiments with plasma clot preparation in which cells were present, it was shown ('15) that a transformation of the clot, and consequently fiber formation, could be brought about by mechanical factors induced by the movements of the cells through the clot. A case of this is shown in figure 5[9] which is a drawing of a portion of a plasma clot preparation at a magnification of 950 diameters in which a piece of muscle tissue had been imbedded. A spindle-shaped cell has left the piece of imbedded tissue and is moving through the fibrin clot. In this preparation, the path of the cell can be traced from the imbedded tissue through the clot to its present position, by means of the fibrillar structure which has arisen in its wake in the ground substance of the clot. In this and similar cases it is clear that the fiber formation in the clot has taken place by the introduction of mechanical factors through the cell movements with a consequent fusion and consolidation of the minute elements of the fibrin clot.

In figure 6 is shown a portion of a transverse section through the tail region of a 75 mm. embryo of R. catesbiana at a magnification of 75 diameters. In this embryo the connective tissue is more mature in type with a practically complete transformation of the ground substance into a fibrous tissue. It will be noted in the figure that the densest formation of connective tissue is in the region immediately surrounding the notochord and also

[9] Baitsell, 1915. A photomicrograph of this preparation is published in this paper as fig. 19.

ventrally in the median line. From the median region of the embryo the connective tissue sheet extends laterally in either direction among the bands of muscle fibers—transverse sections of which are to be seen in the figure—until the body wall is reached. Between the body wall and the muscle tissue there is also a compact layer of the connective tissue. The fibrillation of the connective tissue is heaviest around the notochord. In the region of the muscle tissue the fibrillation is comparatively light and the ground substance closely resembles in its structure that found in young tadpoles as shown in previous figures. Under a higher magnification it will be seen that numerous spindle-shaped cells are scattered in various regions of the preparation lying in cavities on the connective tissue. In some cases they are stretched along the bundles of fibers. Here again the Mallory stain shows a clear differentiation between the cytoplasm of the cell bodies and the common ground substance in which they are imbedded and in no case has it been possible to demonstrate any morphological connection between the cells and the fibers.

DISCUSSION

The researches of Studnicka ('03), Hansen ('05), Mall ('02) and others, upon various phases of connective tissue development in different species of animals, demonstrate conclusively that a rigid adherence to the intracellular theory, in which it is held that the fibers develop directly through a transformation of cell cytoplasm, is no longer possible. The result has been that the intracellular theory as originally stated has been modified by the Hansen-Mall theory which holds, as stated earlier in this paper, that the fibers develop, in general, in a common, living intercellular material, termed the exoplasm, which has been formed as a result of a syncytium of the mesenchyme cells.

At the present time, the inadequacy of the unmodified intracellular theory has been recognized even by Flemming, whose work on the development of connective tissue fibers in the Salamander larvae ('91) is generally regarded as the corner stone of the intracellular theory. Flemming admits that the results

of his researches, in which he showed that the fibers arose through a direct transformation of the cell cytoplasm, do not explain authenticated cases in which it is known that, in the later stages of development of certain connective tissues, an independent growth of the bundles of connective tissue fibers takes place. In such cases Flemming holds ('06) to the Hansen view of a living exoplasm which can nourish itself, grow by intussusception and differentiate new connective tissue fibers.[10]

A review and summary of the question is given by Heidenhain in his Plasma und Zelle ('07). He says[11] that the general opinion of most of the recent investigators is that the first fibrillar differentiation in connective tissue formation arises by a transformation of the cell protoplasm. It is also generally held that a secondary fiber differentiation arises from an organized living matrix independently of cells. Cases are known however (e.g., v. Ebner's work) where the cells apparently first produce a structureless intercellular substance which secondarily produces fibers. With regard to the activities of the organized living intercellular substance (exoplasm), Heidenhain says:[12]

[10] Flemming, 1906, p. 7. To quote: ". . . . ; wie sind aber die späteren Zustände zu denken, wo die schon gebildeten Fibrillen aus der Zelle herausgerückt und Intercellularsubstanz geworden sind? Ich antworte darauf mit Hansen . . . . : dann liegen eben die Fibrillen in dem Territorium von Intercellularsubstanz, das von der betreffenden Zelle geschaffen worden ist, das aus ihrem 'Ektoplasma' (Hansen) hervorgegangen ist; diese Territorien sind mitlebendig, wie ich mir die ganze Intercellularsubstanz so denke . . . . , es können in ihnen Vorgänge fortspielen, die zu einem Längenwachstum der Fibrillen durch Intussusception führen. Ein solches, intussusceptionelles Längenwachstum der Fibrillenbündel wären wir ja übrigens auch genötigt anzunehmen, wenn wir eine freie intercelluläre Fibrillenentstehung voraussetzen wollten. Denn wenn ich mir eine embryonale Sehne in ihren frühesten Zuständen denke und dann spätere dagegenhalte, wo die Bündel 10- und mehrmal so lang geworden sind als zu der Zeit wo sie eben entstanden waren, wie soll das ohne eigene Wachstumverlängerung der Bündel abgegangen sein?" And again (p. 9): "Es bestände danach die gesamte Intercellularsubstanz des Bindegewebes aus solchen vereinigten Ektoplasmen von Zellen, die fibrillär umgewandelt wurden und die, wie ich mit Hansen glaube, mitlebend fortbestehen unter dem vitalen Einflusz der produzierenden Zellen und zur Entwickelung neuer intercellulärer Formteile im stande bleiben."

[11] Heidenhain, 1907, p. 37.

[12] Heidenhain, 1907, p. 37.

Innerhalb der einmal angelegten Intercellularsubstanz wachsen die Bindegewebsbündel späterhin selbständig weiter fort, in die Länge sowohl in die Dicke. Dasz das Längenwachstum ohne eine besondere Tätigkeit der Zellen vor sich geht, ist wohl immer angenommen worden; was das Dickenwachstum anlangt, so war früher die Anschauung vielfach verbreitet, dasz die Zellen neue Fibrillen auf die Oberfläche der erstmals angelegten Bündel abscheiden. Indessen ist dies nur eine Formel, welche zwar aus dem cellularen Prinzip sich ergibt, aber unmöglich überall zutreffen kann. Tatsache ist, dasz man im lockeren Bindegewebe die Zellen oft nur sehr sparsam eingestreut findet, und dasz die Bindegewebsbündel auf weite Strecken hin mit ihnen in keiner Berührung stehen, also nur durch eigene Assimilation wachsen können.

Heidenhain further holds[13] that the living intercellular material is of a more passive nature, in fact a sort of a living framework which lacks many of characteristics of true cell cytoplasm, but still retains at least for a time the power of growth through intussusception, and the ability to differentiate new fibrillar structures. To quote: "Die Metaplasmen sind in Verhältnis zu den eingeschlossenen Zellen eine lebende Substanz besonderer Art, welche in eine andere Bahn der Entwickelung übergegangen ist, ohne dasz eine Möglichkeit der Rückverwandlung in Protoplasma besteht."

The adoption of this view of a living exoplasm with the power to assimilate food material and the ability to differentiate new fibrillar structures independently of the typical cells at once raises a number of questions. In the first place this living exoplasm must be regarded, as stated by Heidenhain, as being of a lower grade of living matter than that found in the regular cell bodies. It possesses only certain of the vital activities and entirely lacks others. In other words, it is necessary to conclude that, in the connective tissues of an animal, there are different degrees of living matter, beginning with the nucleus of the cell as the highest type, then grading into the endoplasm of the cell bodies and finally into the common exoplasm.

In the second place, the postulation of a living exoplasm modifies the cell theory to a great extent, and delegates many of the powers supposedly possessed only by cells to masses of intercellular material, in considerable areas of which even nuclei

[13] Heidenhain, 1907, p. 48.

may be entirely lacking. This raises at once the question as to how such a material is governed. Heidenhain believes[14] that "den Intercellularsubstanzen jene eigenartige Automatie des Lebens zukommt, welche alle lebenden Teile besitzen." He also holds that there is an external regulation of some sort but "Woher diese Regulation stammt, ist uns einstweilen verborgen."

A consideration of the intracellular theory as modified by the studies of Hansen and Mall and the intercellular theory reveals a quite close agreement. This rests upon the recognition in both theories that a common intercellular ground substance is present in which fibers form independently of cells. The difference between the two theories lies in the question of the nature of the intercellular ground substance; the intercellular theory holding that it is a secretion product of the embryonic cells and the Hansen-Mall theory holding that it is a special type of living matter formed by a syncytium of the mesenchyme cells.

In this connection, the present researches give evidence that the ground substance is nothing more than a type of secretion given off by the embryonic cells. This conclusion is based, first, upon the fact that ground substance is formed originally before any syncytium of mesenchyme cells could have been formed and, second, that material which has been properly stained with Mallory's connective tissue stain shows, at all stages, a clear and consistent differentiation in the color reaction between cell cytoplasm and the ground substance.

The ground substance having been formed, the next step we have to consider is the formation of the connective tissue fibers. In this connection from the intracellular standpoint, Flemming and others have clearly demonstrated that the cells in developing connective tissue contain intracellular fibers which they regard as being connective tissue fibers. The presence at times of these intracellular fibrillar structures cannot be questioned but conclusive proof is lacking that they become the connective tissue fibers; there is the possibility that they are transient fibrillations due to various factors, examples of which can be found in many types of cells.

[14] Heidenhain, 1907, p. 48.

In connection with this point, Merkel[15] rightly calls attention to the fact that it is only the formation of the fibers in the first stages of connective tissue development concerning which there is a difference of opinion at present. For a long time there has been no question but that in the later stages of connective tissue development there is a complete separation of fibers and cells. Since this is the case, the logical conclusion appears to be that the fibrillar structures which are present in the connective tissue cells at certain times are not connective tissue fibers, for there is no apparent reason why the fibers in the early stages of connective tissue development should be formed in the cells and later the process change and the fibers be formed in the ground substance independently of cells.

Considerable stress has been laid at times upon the spindle shape of the connective tissue cells or fibroblasts. I regard the shape of the connective tissue cells as almost entirely dependent upon the external factors which the cells encounter. The study of the material shows that when the mesenchyme cells first enter the ground substance they are, in general, rounded, typically-shaped cells. Later, in the ground substance, they assume various irregular shapes, as shown in figure 4, among which will be found a number of spindle-shaped fibroblasts. The idea has been more or less prevalent that cells of the spindle type are in some way 'spinning fibers' and therefore the shape has been regarded as a constant factor for the fibroblasts. The studies of various types of cells in tissue cultures[16] show that the shape of the cell is not a constant factor. Certain cells when grouped together in the cell masses show a typical cell body. The same cells when separated assume various irregular shapes, such as stellate, amoeboid and spindle, depending upon the nature of the medium and whether the cell is exhibiting movement. If the cell begins to move in a certain direction along a fiber or other support it will elongate in a direction parallel to the movement and assume thereby a spindle shape.[17] It is believed,

[15] Merkel, 1908, p. 381.
[16] Harrison, 1914, pp. 535–8. Cf. Figs. 8–12. Matsumoto, 1918, pp. 555 et seq.
[17] Harrison, 1914. Cf. figs. 4–7.

therefore, that the spindle shape of a cell indicates a response to the environment and not a process of fiber formation.

With regard to fiber formation the present work demonstrates that the fibers may develop in regions of the ground substance which are free from cells. At later stages when cells are present the study of the material gives no evidence of any morphological connection between them and the fibers in the ground substance. The complete process of fiber formation from the ground substance can be observed and the transformation into a fibrous tissue appears as a gradual development from a fine fibrillation through various stages until the well developed fibrous condition is reached.

The previous studies of the author, with the plasma clot, show clearly how a morphological transformation of this character may take place, by means of a fusion and consolidation of the minute elements of which the clot is composed, in response to mechanical factors such as tension or pressure. It was suggested at that time[18] that "a reaction of this kind plays an important part in the ontogeny of the individual in the formation of the various connective and supporting structures. The well known fact that they, in general, are laid down in exact correspondence to the definite stresses of the organism leads to the conclusion that in their formation some arrangement must have been present which would respond to the various mechanical factors introduced during development, such as has been shown to be the case with the plasma clot. The generally accepted view of the intracellular origin of the connective tissues does not give any adequate explanation of this fact."

From the morphological standpoint, the results of the present study indicate that the formation of connective tissue in the amphibian embryo is similar to the process which takes place in transformation of the plasma clot. The intercellular ground substance of developing connective tissue may therefore be compared in its morphology to the plasma clot. This ground substance when first formed appears homogeneous or with a

[18] Baitsell, 1917, p. 130.

fine fibrillation. The process of transformation into a fibrous tissue is a progressive one. The fibrillation increases, bundles of fibers are formed and in time the entire ground substance, which at first showed such a high degree of homogeneity, becomes transformed into a fibrous tissue. It is indicated that this transformation occurs as the results of the introduction of mechanical factors in the embryo. These factors may be due to certain lines of tension in the embryo corresponding to the inherent polarity of the organism or, just as in the plasma clot, the movements of the cells through the ground substance may introduce mechanical factors which aid in the transformation of the ground substance into a fibrous tissue. The cells, however, are to be regarded primarily as assimilative and secretive agents, chiefly concerned in the formation of the undifferentiated ground substance.

SUMMARY

1. The primitive forerunner of connective tissue in amphibian embryos is a gelatinous material (primitive ground substance) which can be demonstrated around the notochord soon after it is formed and shortly before the embryo has reached the tail bud stage. A little later this material, which is to form in general the ground substance of the connective tissues, surrounds the medullary cord and a layer of it, following the body wall, extends ventrally on either side and in time completely encircles the body cavity. The formation of this matrix around the notochord takes place before there is any syncytium of the mesenchyme cells in this region. It is evident, therefore, that this primitive ground substance of connective tissue has arisen as an intercellular secretion of the embryonic cells and not by a cytoplasmic syncytium.

2. The ground substance having been formed, cells begin to move into it and wander through it. These are spherical at first, but as they move through the ground substance, they soon change into various shapes, becoming stellate, spindle-shaped, etc. The study of the sections shows that, in general, individual cells do not separate from the cell masses and move out into the

various cavities and open spaces of the embryo until after the formation of the secreted ground substance which is the forerunner of the connective tissues. It appears evident therefore that cells, whether in tissue cultures as shown by Harrison[19] or in the developing embryo, have need of a supporting framework of some kind in order for movement from the main masses to take place.

3. The connective tissue fibers begin to arise in the ground substance soon after it has formed. In some cases, they form in the ground substance in regions which are free from cells so that it is certain that they have not arisen by an intracellular action. The fibers are evidently formed by a gradual transformation of the ground substance, first, into a delicate net-like structure and then into the long fibers which are typical of connective tissue. In its morphological features, at least, this process gives evidence of being identical with the one previously observed in the transformation of the plasma clot.

4. In those regions of the ground substance into which the cells have wandered, the formation of the fibers can also be shown to be due to changes in the ground substance and not to a sloughing off of the cell protoplasm. A differential stain, such as Mallory's connective tissue stain, shows definitely the boundaries of the cell cytoplasm and of the ground substance. It is suggested that the movements of the cells through the ground substance introduce mechanical factors which aid in fiber formation just as has been shown to be the case in plasma clots. In any case, the fact that the fibers can form in a cell-free ground substance shows that no part of the cell cytoplasm is necessary for their differentiation from the ground substance; nor is it possible to demonstrate any morphological connection between the cells and the fibers in the ground substance.

[19] Harrison, 1914, pp. 540 and 543.

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For complete bibliographies of this subject see references to Heidenhain, Flemming or Merkel above.

PLATE 1

EXPLANATION OF FIGURES

1 Portion of sagittal section of 3.2 mm. embryo of R. palustris. × 285. The notochord (N.C.) is shown imbedded in the cell-free connective tissue ground substance (G.S.). The latter extends laterally on either side into the muscle plates.

2 Portion of transverse section of 4 mm. embryo of R. palustris. × 322. A section of the notochord (N.C.) is shown imbedded in the connective tissue ground substance (G.S.).

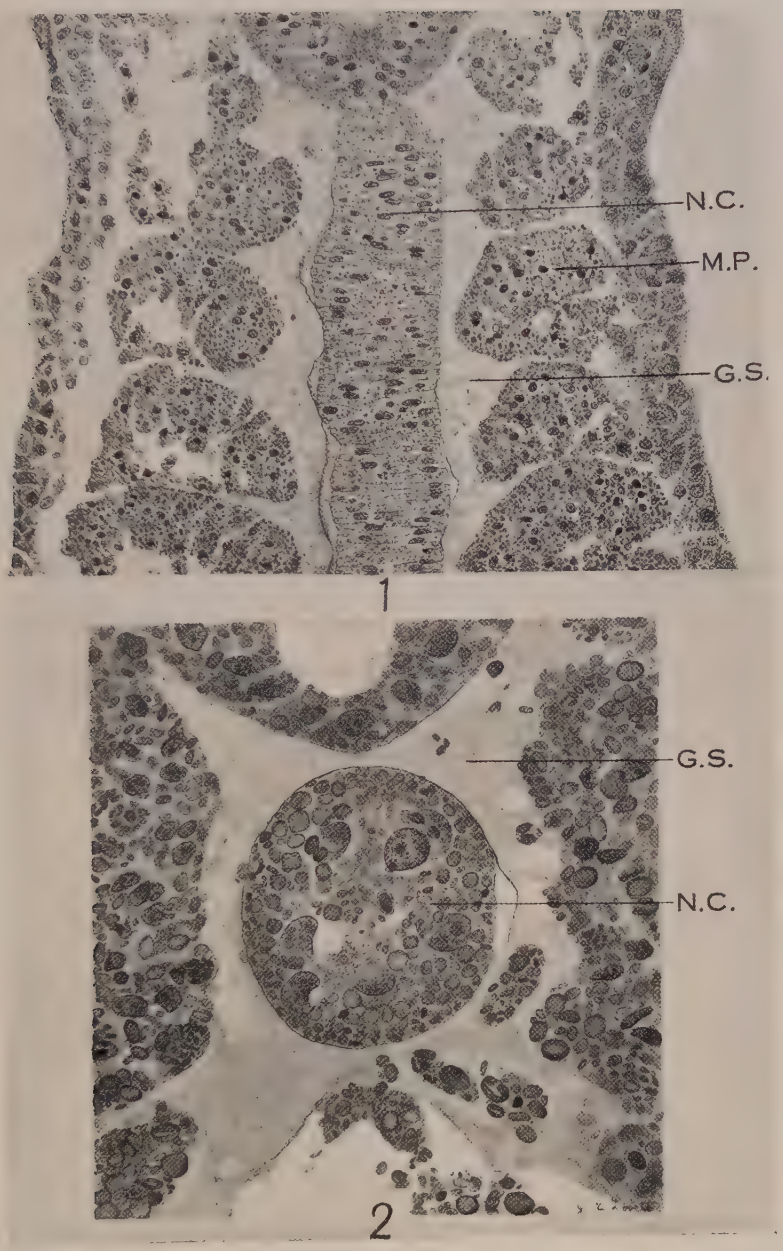
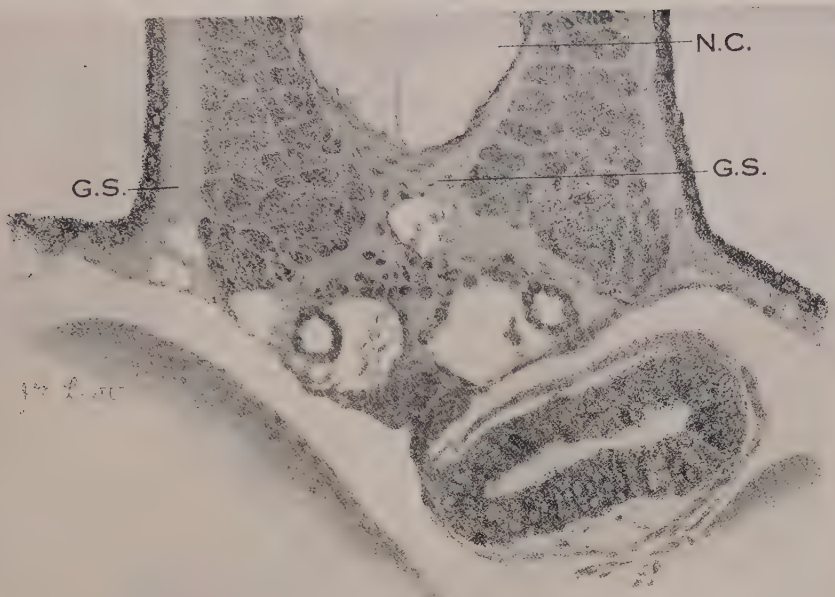

N.C.
M.P.
G.S.
1
G.S.
N.C.
2

PLATE 2

EXPLANATION OF FIGURES

3 Portion of 9 mm. embryo of R. sylvatica. × 322. The ground substance (G.S.) is shown lying below the notochord (N.C.) and extending laterally on either side between the muscle plates to the body wall. At this stage some of the mesenchyme cells are beginning to move into the ground substance.

6 Portion of a transverse section through the tail region of a 75 mm. embryo of R. catesbiana. × 75. The figure shows a portion of the notochord surrounded by the connective tissue ground substance (G.S.). In this late stage the ground substance, particularly around the notochord (N.C.), is largely composed of bundles of typical connective tissue fibers.



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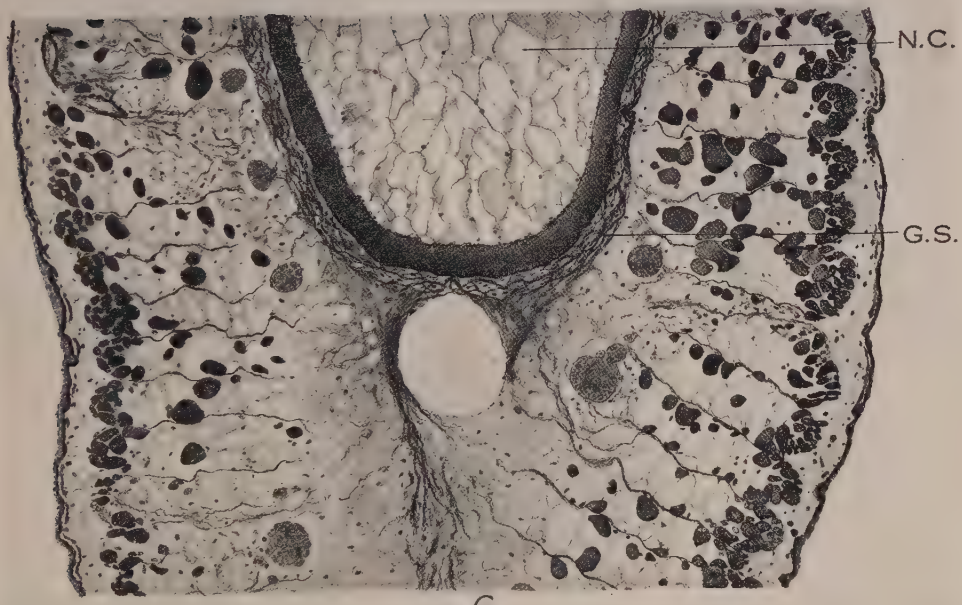

6

PLATE 3

EXPLANATION OF FIGURES

4 Portion of transverse section of 11 mm. embryo of R. sylvatica. × 760. Mallory connective tissue stain. The region shown lies just below the notochord as in figure 3. The connective tissue ground substance (G.S.) contains a number of wandering mesenchyme cells which take the same stain as do the surrounding muscle fibers (M.F.)—both types of cells being clearly differentiated by the stain from the secreted connective tissue ground substance which is formed before any cells are present in it.

4

PRESS WORK BY FRED'K GOEB

PLATE 4

EXPLANATION OF FIGURE

5 Section of plasma clot from a tissure culture. × 950. The figure shows the transformation of the fibrin net (F. N.) of a plasma clot into a fibrous tissue (F. T.) as a result of the mechanical factors induced by the movements of the cell through it. Cf. Baitsell, 1915, fig. 19.

5

PRESS WORK BY FRED E GOEB

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1921, xviii, pp. 172–173.

84 (1666)

The use of blood plasma in the imbedding or the dissection of small organisms.

By GEORGE A. BAITSELL.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Various methods have been used in order to overcome the difficulties attendant upon the carrying of a minute organism, such as a protozoön, through the numerous reagents necessary for imbedding in paraffine preliminary to microtome sectioning. It has been found that the phenomenon of coagulation in blood plasma makes it of use in work of this type. In carrying out this method, a few drops of blood plasma is placed on a depression slide under a binocular microscope and the specimen at once placed in it with a pipet. After the plasma coagulates, which will take place in a very few minutes, the specimen will be found to be firmly imbedded in a resistant fibrin clot which can be taken through the various reagents, imbedded in paraffine, sectioned in any desired plane and finally mounted on a slide. In brief, the clot containing the specimen may be treated as a regular piece of tissue of the same size.

The orientation of the specimen in the clot may be accomplished before the clot is fully formed or at the time when the clot is in the clearing fluid just prior to imbedding. In the clearing fluid the clot becomes transparent and can be examined under a microscope and the imbedded specimen located in it. The clot can then be trimmed so as to indicate the orientation of the specimen.

Plasma of various animals can be used. I have generally used frog plasma and have secured it by the method previously described.[1]

Blood plasma has also been found to be useful in holding small animals firmly in a certain position so that they can be dissected. In this connection it may be noted that the dissection can proceed as far as desired and then no matter how fragile

[1] *Journal of Experimental Medicine*, 1915, xxi, 456.

the dissected parts may be, additional plasma can be added and the fragile dissected part or parts imbedded in a clot in the same manner as described above. If desired, the clot can then be taken through the regular reagents and imbedded in paraffine for sectioning.

There is no doubt that blood plasma can be put to many other similar uses depending upon the nature of the problems and the ingenuity of the investigator. The securing and keeping of the plasma will not be found too difficult after the operation has been performed a few times.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1921, xviii, pp. 188–189.

93 (1675)

The life history of an amicronucleate race of Didinium nasutum.

By MARY W. PATTEN.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

A series of pedigree lines of *Didinium nasutum*, consisting of the progeny of a single exconjugant, were bred continuously under practically constant environmental conditions from December 16, 1919, to September 10, 1920.

A cytological investigation of the preparations made daily from stock left after each isolation, demonstrated the absence of a morphological micronucleus in vegetative, dividing and conjugating animals. In the race from which these pedigree lines were derived by conjugation, and also in other unrelated races of *Didinium*, micronuclei were easily demonstrable. It is clear, therefore, that the parent race was micronucleate, while one of its progeny, a single exconjugant (the original cell of the race under investigation) was amicronucleate. Moreover, this amicronucleate condition persisted throughout the life of the race, that is, through 652 generations.

At various intervals during the life history of this culture, periods showing a tendency for encystment and conjugation occurred, but the animals which encysted or conjugated invariably died, a fact undoubtedly related to their amicronucleate condition. Rhythmical periods of depression followed by increased vitality, which resembled those shown by Woodruff and Erdmann[1] to be associated with endomixis in *Paramecium caudatum* were also observed. Endomixis, however, if such occurs in the free living state of *Didinium*, could not be carried out in this race during the rhythmical periods owing to the lack of a definitive micronucleus.

The amicronucleate state without doubt arose from some irregularity during conjugation. Prandtl[2] in his study of the

[1] Woodruff, L. L., and Erdmann, R., "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exp. Zoöl.*, 1914, xvii, 425–518.

[2] Prandtl, H., "Die Konjugation von *Didinium nasutum*," *Arch. f. Protistenkunde*, 1906, vii, 229–258.

cytology of conjugation in *Didinium* found individuals in which all the micronuclei had become transformed into macronuclei, but he thought animals so endowed were incapable of living. The present study shows that they are viable but unable to undergo processes such as encystment, conjugation, and endomixis which are dependent largely upon micronuclear activity.

[Reprinted from The American Naturalist, Vol. LV., Mar.–Apr., 1921.

STANDARDIZED MICROPHOTOGRAPHY

Second Contribution: The Object Factor

In my first contribution to the subject of standardized microphotography, published in the *Anatomical Record*, I have pointed out the variables and the methods which I have pursued in treating them. One, or perhaps more correctly a group of variables were, however, left out of consideration quite purposely because of the difficulty in finding for them a standard of permanent value. I have in mind the microscopical section itself or what may be properly called the object factor. The following four elements enter into its composition: (1) the thickness of the section, (2) the light absorption coefficient of the tissue, (3) the relative luminosity of the different stains and (4) the depth or intensity of staining. The second and third of these component elements may be disregarded since experience shows us that exposure is very little influenced by them. There remain, however, the first and fourth, and to determine the influence of these on exposure the following experiments were undertaken.

First of all, to avoid all possible error, slides were chosen of uniform thickness measured with a Ciceri Smiths Patent Micrometer so as to be sure that the distance of the section from the substage condenser should in every case be the same. The cover-glasses were also of uniform thickness. The stomach of a frog preserved in Zenker's fixing fluid was sectioned into series of 5, 10 and 20 micromillimeter thick sections on a Minot microtome and care was taken to have in each case a ribbon of 100 even sections, thus more or less assuring their uniform thickness. The sections were stretched on distilled water heated over a flame and no cement of any kind was used. On one slide three sections of each thickness were placed. On other slides sections only of one kind were placed and their thickness marked in every case by a carborundum pencil.

The slide with all three kinds of sections was stained for 12 hours in alumcarmine, a stain which, as is well known, does not overstain. They were then washed in water and again stained for 12 hours in a weak alcoholic solution of Bleu de Lyon. All sections on this slide received therefore the same treatment and the difference in the depth of stain was entirely due to the thickness of the section stained.

The other slides were treated in a different way. They were stained in Delafield's hæmatoxylin followed by tetrabromfluorescic acid. This stain was chosen because it is possible at will to control the depth of staining. A set A of three slides, one with 5 micromillimeter sections, one with 10 and one with 20, was treated simultaneously in a Coplin's staining jar. The sections were therefore stained, washed, destained in acid alcohol, treated with ammonia alcohol, stained in a weak solution of tetrabromfluorescic acid in 95 per cent. alcohol and washed in pure alcohol the same length of time. In regard to depth of stain these slides presented, therefore, the same conditions as the slide stained in alumcarmine and Bleu de Lyon.

Several other slides were treated individually in the same stains. They were all first considerably overstained in Delafield's hæmatoxylin, washed in water and destained in acid alcohol until they had when viewed over a white surface, the same shade of color to the naked eye, regardless of the thickness of the section. If after treatment with ammonia alcohol the blue color was not approximately of the same shade, the darker slide was again transferred to the acid alcohol, until all sections looked approximately alike. They were now stained in the tetrabromfluorescic acid, the thickest sections remaining a short time in the fluid, the 10 micromillimeter sections somewhat longer and the 5 micromillimeter sections longest, and again compared over a white surface. From several slides thus prepared three were selected which to the naked eye were to all purposes of the same appearance, although their respective thickness were 5, 10 and 20 micromillimeters. Here then we had a Set B of sections in which the depth of stain had nothing to do with the thickness of the section, but was entirely dependent upon the amount of stain absorbed by the tissues.

Fractional exposures on orthonon plates with Cramer ray-filters were made on our standardized microphotographic apparatus and developed by the time and temperature method with

fresh developer for each plate. In the case of the first slide stained in alumcarmine and Bleu de Lyon as in the Set A, the normal exposure for the 10 micromillimeter section was twice that for the 5 one, and the normal exposure for the 20 micromillimeter section was four times that of the 5 one. In the case of the Set B all slides required the same exposure.

In analyzing the results thus obtained we come to the conclusion that the thickness of the section, within the limits given, has no influence whatsoever on the length of exposure; but that the latter stands in a direct ratio to the amount of stain absorbed by the tissue.

For practical purposes, especially for those who are using my table of R-P factors, the results of this investigation may be interpreted as follows: disregard the thickness of the sections and the appearance of the stain under microscope and pay attention only to the intensity as it appears to the naked eye. The table refers to normally well stained sections of medium thickness. Double the exposure for darker appearing and reduce the exposure by half for lighter appearing sections.

It will be observed that in the experiments elements 2 and 3 remained of constant value for the simple reason that the tissue used was not only the same in kind, but actually from the same piece of organ and was stained in the same stain in each set of slides. A comparison of the length of normal exposure in the case of sections stained in alumcarmine and Bleu de Lyon with those stained in hæmatoxylin and tetrabromfluorescic acid serves to confirm my statement at the beginning of this article, that the relative luminosity of different stains may be entirely disregarded in the matter of exposure.

ALEXANDER PETRUNKEVITCH

YALE UNIVERSITY.

[Reprinted from The Scientific Monthly, March, 1921]

HISTORY OF BIOLOGY*

By Dr. LORANDE LOSS WOODRUFF
PROFESSOR OF BIOLOGY, YALE UNIVERSITY

SOME practical acquaintance with plants and animals undoubtedly formed the chief content of the mental equipment of prehistoric man, and a considerable knowledge of agriculture and medicine was possessed by the Egyptians and Sumerians nearly 7,000 years ago. So biology has a very ancient pedigree. But biology as the science of life—the study of living phenomena for their own sake in which emphasis is shifted from the practical to the philosophical—really begins with the Greeks, and reaches per saltum a height which was not surpassed, indeed not again attained, for nearly twenty centuries.

Science reaching Greece from the South and East fell upon fertile soil, and in the hands of the Hellenic natural philosophers was organized into coherent systems through the realization that nature works by fixed laws—a conception foreign to the Oriental mind and the cornerstone of all future work because it gave purpose to personal scientific investigation. This attitude of approach is largely responsible for the transformation of the Greek scientific heritage from a collectivistic to an eponymous product.[1] It is not an exaggeration to say that to all intents and purposes the Greeks laid the foundations of the chief subdivision of natural science and, specifically, created biology, though the term biology was first used by Lamarck and Treviranus at the beginning of the nineteenth century.[2]

Aristotle, (384-322 B. C.) the most famous pupil of Plato and dissenter from the Platonic School, represents the highwater mark of the Greek students of nature and is justly called the Father of Natural History. Aristotle's contributions to biology are manifold. He took a broad survey of the existing facts and welded them into a science by relying, to a considerable extent, on the direct study of organisms and by insisting that the only true path of advance lay in accurate observation and description. But mere observation without interpretation is not science. Aristotle's generalizations—his elaboration of broad philosophical conceptions of organisms give to his biological works their perennial significance. Among the facts and supposed facts, and

*Delivered at Yale University, April 29, 1920; the third lecture of a series on the History of Science, given under the auspices of Yale Chapter of the Gamma Alpha Graduate Scientific Fraternity.

[1]E. Clodd: Pioneers of Evolution, 2d ed., 1907, pp. 29-32. C. Singer: Studies in the History and Method of Science, 1917.

[2]Lamarck: Hydrogéologic, 1802. G. R. Treviranus: Biologie, oder Philosophie der lebenden Natur für Naturforscher und Aerzte, 1802-22.

of course there are innumerable crudities if for no other reason than that adequate apparatus and biological technique were of the distant future, there are interspersed questions, answers, theories which show a recognition and remarkable grasp of fundamental biological problems. A study of Aristotle's works shows ancient pedigrees for some of the most "modern" questions of biology, though it is undoubtedly true, as Sachs insists, that one must continually inhibit the tendency to read the present viewpoint into the past, and not assign to earlier writers merits which, if they were alive, they themselves would not claim.

We have not mentioned a single discovery made by Aristotle—and with purpose. Aristotle's position as the founder of biology rests chiefly on his viewpoint and his methods. Plato relied on intuition as the basis of knowledge. Aristotle emphasized observation and induction, insisting that errors arise not from the false testimony of our sense organs but from false interpretations of the data they afford. "We must not accept a general principle from logic only, but must prove its application to each fact; for it is in facts that we must seek general principles, and these must always accord with facts from which induction is the pathway to general laws."[3] But it is not to be imagined that Aristotle always followed his own advice; few great men do—"no pilot can explore unsurveyed channels without a confidence which sometimes leads to disaster." It must be admitted that Aristotle frequently lapsed into unbridled speculation which tended to obscure the methods that time has shown produce the most enduring results, though, as Huxley has well said, "It is a favorite popular delusion that the scientific enquirer is under a sort of moral obligation to abstain from going beyond that generalization of observed facts which is absurdly called 'Baconian' induction. But any one who is practically acquainted with scientific work is aware that those who refuse to go beyond fact, rarely get as far as fact; and any one who has studied the history of science knows that almost every step therein has been made by the 'Anticipation of Nature,' that is, by the invention of hypotheses, which, though verifiable, often had very little foundation to start with; and not infrequently, in spite of a long career of usefulness, turned out to be wholly erroneous in the long run."[4]

While Aristotle's biological investigations were devoted chiefly to animals, his pupil and co-worker, Theophrastus (370-286 B. C.), made profound studies on plants, and the list of botanical facts which he observed and in many cases discovered includes nearly all the rudi-

[3]Aristotle: History of Animals, I, 6. H. F. Osborn: From the Greeks to Darwin, 1894, pp. 16-17, 47. T. H. Huxley: On Certain Errors Respecting the Heart Attributed to Aristotle, 1879 (Coll. Sci. Memoirs, IV, pp. 380-392); G. H. Lewes: Aristotle; a Chapter in the History of Science, 1864, pp. 108-13. T. E. Jones: Aristotle's Researches in Natural Science, 1912. W. Whewell: The Philosophy of Discovery, 1860.

[4]T. H. Huxley: The Progress of Science, 1887 (Coll. Essays, vol. 1, p. 62.)

ments of scientific botany. It is a remarkable fact that many details in plant anatomy, which were figured by the pioneers with the microscope, are to be found in the pages of Theophrastus.[5] He not only laid the foundations of botany, but also gave suggestions of much of the superstructure; an achievement which entitles him to rank as "the first of real botanists in point of time."[6]

With the Greeks, then, biology emerged from the shadows of the past and took concrete form—a fact which apparently the discerning mind of Aristotle appreciated since, though frequently referring to the ancients, he writes:

> I found no basis prepared; no models to copy. . . . . Mine is the first step, and therefore a small one, though worked out with much thought and hard labor. It must be looked at as a first step and judged with indulgence.

Before leaving the Greeks we must mention Hippocrates (460-370 B. C.), the Father of Medicine. Writing a generation before Aristotle, at the height of the Age of Pericles, Hippocrates crystallized the knowledge of medicine into a science, dissociated it from philosophy, and gave to physicians "the highest moral inspiration they have." "To him medicine owes the art of clinical inspection and observation, and he is, above all, the exemplar of that flexible, critical, well-poised attitude of mind, ever on the lookout for sources of error, which is the very essence of the scientific spirit. . . . . The revival of the Hippocratic methods in the seventeenth century and their triumphant vindication by the concerted scientific movement of the nineteenth, is the whole history of internal medicine."[7]

Medicine, the most important aspect of applied biology, is the foster parent of zoology and botany since a large proportion of biological advances have been the work of physicians. Until relatively recently the schools of medicine afforded the only training, and the practice of medicine the chief livelihood for men interested especially in general biological problems.[8] The history of medicine and of biology, as a so-called pure science, are so inextricably interwoven that the consideration of one involves that of the other. Indeed the physicians form the only bond of continuity in biological history between Greece and Rome. The chief interest of the Romans lay in technology, and therefore it is natural that the practical advantages to be gained would ensure the advance of medicine.[9] As it happens, however, two Greek physicians were destined to have the most influence: Dioscorides, (C. 64 A. D.), an army surgeon under Nero, and Galen (131-201 A. D.), physician to the Emperor Marcus Aurelius and his son, Commodus.

Just as Theophrastus established botany as a pure science, so

[5]E. L. Greene: Landmarks of Botanical History, 1909, pp. 52, 53, 140-142.
[6]A. Haller: Bibliotheca Botanica, I, 31.
[7]F. H. Garrison: History of Medicine, 2d ed., 1917, p. 82.
[8]T. H. Huxley: The Connection of the Biological Sciences with Medicine. Nature, 24, 1881, pp. 342-46; (Also in Coll. Essays).
[9]W. Libby: An Introduction to the History of Science, 1917, Chapter 3.

Dioscorides was the originator of the pharmacopoeia, writing, as he did, not only a work which was the first one on medical botany, but one which, gaining authority with age, was the sole standard "botany" for fifteen centuries. Theophrastus was long overshadowed. Most of the botanical writings up to the seventeenth century were annotations on the text of Dioscorides.[10]

Galen was the most famous physician of the Roman Empire and his voluminous works represent both the depository for the anatomical and physiological knowledge of his predecessors, rectified and worked over into a system, and a vast amount of original investigation. Galen was a practical anatomist who described from dissections and insisted on the importance of vivisection and experiment, and therefore may be considered the first experimental physiologist and the founder of experimental medicine. Galen gave to medicine its standard anatomy and physiology for fifteen centuries.[11]

Any consideration of the biological science of Rome would be incomplete without a reference to the vast compilation of fact and fiction, indiscriminately mingled, made by Pliny the Elder (23-79). It was beside the path of biological advance, but long the recognized "Natural History," passing through some eighty editions after the invention of printing. Its prestige was largely due to the fact that it was written in Latin whereas the great works on biological subjects were in Greek.[12]

For all practical purposes we may consider that biology at the decline of the Roman Empire was represented in the works of Aristotle, Theophrastus, Dioscorides, Galen, and Pliny. Even these exerted little influence during the Middle Ages, being saved from total loss for future generations chiefly by Arabian scientists, and in the monasteries of Italy and Britain. We cannot pause to consider the various causes which resulted in the almost complete break in the continuity of learning in general and science in particular during the dormant period in western Europe.[13] Suffice it to say that contributing factors were wars and rumors of wars, the destruction of the libraries of Alexandria, the antagonism of Christian and pagan ideals, and the establishment by the Church, which held the gates of learning, of the written word in place of observation of nature as it is. "Truth and science came to mean simply that which was written, and inquiry became mere interpretation."

In so far as science was taught at all it was from small compilations of corrupt texts of ancient authors interspersed with anecdotes and fables. Under theological influence there arose the oft-quoted

[10]Greene, op. cit., pp. 151-154.

[11]M. Foster: Lectures on the History of Physiology, 1901. Garrison, op. cit., p. 97-101. M. Verworn: General Physiology, English trans., 1899, pp. 8-11.

[12]F. E. Hulme; Natural History Lore and Legend, 1895, pp. 20-29. Greene, op. cit., pp. 155-159.

[13]A. D. White: History of the Warfare of Science with Theology in Christendom, 1898. Clodd, op. cit., p. 34. Russell, op. cit., pp. 124 et seq,

Physiologus,[14] found in many forms and languages, which is at once a collection of natural history stories, and a treatise on the medicinal use of animals and on symbolism. The centaur and the phoenix take their place with the frog and crow in affording illustration of theological texts and in pointing out more or less evident morals. The line of demarkation between the Physiologus and the Bestiaries into which it gradually evolved is ill defined, while the remnants of the latter are incorporated in the early works of the Renaissance encyclopaedists.[15]

So low had science fallen that, strange to say, the scientific Renaissance may be said to owe its origin to the revival of classical learning and to the translation and study of the writings of Aristotle and others which had been under eclipse for a millennium. These were so superior to the existing science, if it may be dignified by that name, that, in accord with the spirit of the time, Aristotle and Galen became the bible of biology. The first works were merely commentaries on the classical authors, but as time went on more and more new observations were interspersed with the old until elaborate and voluminous treatises describing all known forms of plants and animals were produced. In short, the climax of the scientific Renaissance involved a turning away from the authority of Aristotle and an adoption of the Aristotelian method of observation and induction.

Botany was the first to show visible signs of the awakening, probably because of the dependence of medicine on plant products. "All physicians professed to be botanists and every botanist was thought fit to practice medicine." The Hortus Sanitatis, in a way the botanical counterpart of the Bestiaries, gave place to the Herbals.[16] At the hands of the Herbalists of Germany during the sixteenth century, such as Brunsfels, Tragus, Fuchs, and Valerius Cordus, we can trace the evolution of plant description and classification from mere annotations on Dioscorides to well illustrated manuals of the flora of western Europe.[17]

During the same century zoology made abortive attempts to emerge as a science, but the less immediate utility of the subject combined with the difficulty of collecting material and therefore the necessity of more dependence on traveler's tales, all contributed to retard its advance.

One group of naturalists, the Encyclopaedists, so-called from their endeavor to gather all possible information of living things, attempted the impossible. Gleaning from the ancients and adding such materials as they could collect led to the production of huge books of fact and fancy whose value bore no just proportion to the vast expenditure of labor, even in the case of the best—Gesner's Historia Animalium,

[14]White, op. cit. Also, F. Lauchert: Geschichte des Physiologus, 1889.
[15]Hulme, op. cit., pp. 31, 50.
[16]A. Arber: Herbals; their Origin and Evolution. A Chapter in the History of Botany. 1912.
[17]Greene, op. cit., pp. 164 et seq.

which appeared volume by volume between 1551 and 1587, and comprised some 4,500 folio pages of text and wood cuts.[18]

Although Gesner (1516-1565) was without doubt the most learned naturalist of the period and perhaps the best zoologist that had appeared since Aristotle, the direct path to progress was blazed by men whose plans were less ambitious than those of the Encyclopaedists. Thus, contemporaries of Gesner, men who were befriended by him, such as Rondelet (1507-1566) who gave descriptions of the fishes of the Mediterranean based for the most part on his own observations, and Belon (1517-1564) who illustrated the fishes and birds which he saw in France and the Levant, really instituted the zoological monograph which has proved the productive method of biological study.[19]

Even while the Herbalists, Encyclopaedists and Monographers were at work in natural history, making brave attempts to develop the powers of independent judgment which were oppressed to such an extent during the Middle Ages that the very activity of the senses seemed stunted, the emancipator of biology from the thraldom of the ancients appeared in the Belgian anatomist Vesalius (1514-1564). Disgusted with the anatomy of the time which consisted almost solely in interpreting the works of Galen by reference to crude dissections made by barber's assistants, Vesalius set his hand to the task of placing human anatomy on the firm basis of exact observation, and his great work De Humani Corporis Fabrica makes the year 1543 the dividing line between ancient and modern anatomy.[20] Galen's similar attempt failed because his followers made a bible of his work; but with Vesalius, the time was opportune and, in spite of the opposition of his former teacher Jacobus Sylvius and his pupil Columbus, thenceforth anatomical as well as biological investigation in general broke away from the yoke of authority and men began to trust their own eyes. His successor at Padua, Fallopius, says that Vesalius "so shewed me the true path of inquiry that I was able to walk along it still farther than had been done before."

The work of Vesalius is on anatomy, and physiology is treated somewhat incidentally, though it is evident that he was no better satisfied with Galenic physiology than with Galenic anatomy. The complementary work on the side of function came in 1628 with the publication of Harvey's tract, Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus. No rational conception of the economy of the animal organism was possible under the influence of the Galenic system, and it remained for Harvey (1578-1657) to demonstrate by a series of experiments, logically planned and ingenuously executed,

[18]L. C. Miall: The Early Naturalists, their Lives and Work, 1912, pp. 47-50.
[19]Miall, op. cit. pp. 40-47.
[20]W. Stirling: Some Apostles of Physiology, 1902, pp. 2-5. M. Foster, op. cit., p. 2.

that the blood flows in a circle from heart back to heart again, and thus to supply the background for a proper understanding of the dynamics of the organism as a whole. A new picture of the function of the blood was presented which quickly led to the discovery of the lymphatic system, and gave content to the study of the nutrition of the body.[21]

Harvey's use of distinctively quantitative factors is so important in its establishment of the experimental method in biology that his own statement is of great historical interest:

> I frequently and seriously bethought me, and long revolved in my mind, what might be the quantity of blood which was transmitted, in how short a time its passage might be effected, and the like; and not finding it possible that this could be supplied by the juices of the ingested aliment without the veins on the one hand becoming drained, and the arteries on the other hand getting ruptured through the excessive charge of blood, unless the blood should somehow find its way from the arteries into the veins, and so return to the right side of the heart; I began to think whether there might not be a motion, as it were, in a circle. Now this I afterwards found to be true; and I finally saw that the blood, forced by the action of the left ventricle into the arteries, was distributed to the body at large, and its several parts, in the same manner as it is sent through the lungs, impelled by the right ventricle into the pulmonary artery, and that it then passed through the veins and along the vena cava, and so round to the left ventricle in the manner already indicated. Which motion we may be allowed to call circular.[22]

With the work of Vesalius and Harvey, biologists had again laid hold of the great scientific tools—observation, experiment, induction, which since have not slipped from their grasp.

The Microscopists

Even while the marshalling of accurate descriptions of plants and animals was getting under way, and the study of microscopic anatomy and physiology was making rapid strides forward, an event occurred which was destined to make possible modern biology. This was the adaptation of the principle of the spectacles—the invention, probably by Roger Bacon, of convergent lenses, and therefore of the simple microscope. Then came the compound microscope as a development of the telescope at the hands of Galileo about 1610, and by the end of the century simple and compound microscopes were being made by opticians in the leading centers of Europe.[23]

The earliest clear appreciation of the importance of studying nature with instruments which increase the powers of the senses in general and the vision in particular, is found in a remarkable book, the "Micrographia" of Robert Hooke (1635-1703), published by the Royal Society of London in 1665, which is a demonstration of the advantages to be gained by the use of artificial devices of precision in studying

[21] J. G. Curtis: Harvey's Views on the Use and Circulation of the Blood, 1915. T. H. Huxley: William Harvey, Fortnightly Review, 23, 1878 (Also Coll. Sci. Mem., Vol. 4, p. 319).

[22] Harvey: De Motu Cordis et Sanguinis, English trans. by R. Willis, 1848. (See reprint in "Everyman's Library," pp. 55-56.)

[23] Miall, op. cit., p. 136.

nature. "The next care to be taken, in respect to the senses, is a supplying of their infirmities with instruments, and, as it were, the adding of artificial organs to the natural; this in one of them has been of late years accomplished with prodigious benefit to all sorts of useful knowledge, by the invention of optical glasses. . . . . It seems not improbable, but that by these helps the subtiliety of the composition of bodies, the structure of their parts, the various texture of their matter, the instruments and manner of their inward motions, and all the other possible appearances of things, may come to be more fully discovered. . . . . ."[24]

Although the work is replete with singular anticipations of the discoveries and inventions of other workers in various branches of science, the biologist's interest is chiefly in Hooke's application of his improved compound microscope to the study of plants and animals which paved the way for the more special, profound, and methodical studies of the contemporary students of nature. In the Micrographia are clearly described and figured for the first time the "little boxes or cells"[25] of organic structure, and his use of the word "cell" is responsible for its application to the protoplasmic units of modern biology. It is fair to say that the influence of the Micrographia permeated the sciences in various directions and the illustrations of microscopic objects were copied for nearly two centuries.[26]

The Micrographia was a mere incident in the varied interests of Hooke, while van Leeuwenhoek (1632-1723) spent a long life in studying nearly everything which he could bring within the scope of his simple lenses. Fascinated with the "world of the infinitely little," with a virgin field before him, and no literature on the subject to divert his energies, he was able year after year to send letters to learned academies, chiefly the Royal Society of London, describing what of necessity were discoveries. He saw the Bacteria—he is the first bacteriologist—the Infusoria—he is the first protozoologist—Yeast cells, Rotifers, Hydra; but the list is too long.[27] However, all of Leeuwenhoek's work is by no means dusultory. He displayed much ingenuity in studying the flow of blood through capillaries of the web of the frog's foot and bat's wing, the viviparous reproduction in aphids, the development of fleas, the dessication of rotifers. And all the time he was looking for evidence against the idea of spontaneous generation, being convinced "it is fully proved that no living creature is produced by corruption or putrefaction."[28] But Leeuwenhoek's contribution which attracted the most interest was his description of spermatozoa. Brought to his attention by a young physician,

[24]Micrographia, preface.
[25]Micrographia, observation XVIII, pp. 112-116.
[26]L. L. Woodruff: Hooke's Micrographia, American Naturalist, 53, 1919.
[27]Miall, op. cit., pp. 200-223. B. W. Richardson: Disciples of Aesculapius, 1900.
[28]Select Works of A. van Leeuwenhoek, edited by S. Hoole, 1800-7.

Hamm, Leeuwenhoek transmitted the discovery to the Royal Society. His imagination, however, outstripped his observations and he thought he saw evidence of a homunculus within the spermatozoon. Thus he came to regard the sperm as the true germ which had only to be hatched, as it were, by the female. He was thus the first of the school of 'spermists' which opposed the idea of the 'ovists' that all is performed in the egg.[29]

The patience of Leeuwenhoek would have been strained to the breaking point by the studies on insect anatomy made by Swammerdam (1637-1680) and eventually brought together in his Biblia Naturae. Instigated largely by the desire to refute the current notion, supported by certain statements of the great Harvey, that insects and similar lower animals are merely masses of organic matter which have been moulded, as it were, into a definite form, and are without complicated internal organs, Swammerdam spent his life in studies on their structure and life histories.[30] Revealing as he did, by the most delicate technique in dissection, the finest details observable with his lenses, Swammerdam not only set a standard for minute anatomy which, with that of his contemporary Malpighi, was not surpassed until the work of Lyonet and others nearly a century later, but also dissipated, for all time, the conception of simplicity of structure in the lower animals. Swammerdam thus quite naturally added one more argument to those of Redi and others against spontaneous generation:

> That vulgar opinion, . . which ascribes birth and growth of animals to putrefaction and chance, is diametrically opposite to sound reason. . . . in the smallest animals we constantly everywhere find as much order, contrivance, beauty, wisdom, and omnipotence in the Great Architect, as are shown in the viscera of the largest animals. For to these greater animals all others, however minute, are similar in the great respects of brain, nerves, muscles, heart, stomach, intestines, and parts subservient to generation, and to every other useful purpose; so that one might in a manner affirm, that God has created but one animal, though divided into an infinite number of kinds or species, differing from each other in the figures and inflexions, and extensions of their limbs, as likewise in their dispositions, food and manner of living.[31]

Contemporaries of Hooke, Leeuwenhoek, and Swammerdam were two men who may be considered as the pioneer histologists—Malpighi of Bologna and Grew of London. Grew (1641-1712) devoted all his attention to plant structure, while Malpighi, in addition to botanical studies which paralleled Grew's, made elaborate investigations on animals.

The versatility as well as the genius of Malpighi (1628-1694) is illustrated by his studies on the anatomy of plants, the function of leaves, the development of the plant embryo, the embryology of the chick, the anatomy of the silkworm, the structure of glands.[32] Skilled

[29]Phil. Trans. Royal Soc., 142, 1678.
[30]H. Boerhaave: Life of Swammerdam (Preface to the Biblia Naturae) F. C. Miall, op. cit., pp. 174-199. Also Miall's History of Biology, 1911.
[31]Biblia Naturae, English trans. Edited by John Hill, 1758, part 2, p. 71.
[32]Miall, op. cit., p. 145.

in anatomy but with prime interest in physiology, his lasting contribution lies in his dependence on the microscope for the elucidation of problems where structure and function, so to speak, merge—well illustrated by his ocular demonstration of the capillary circulation in the lungs; at once his first and greatest discovery and the first of prime importance ever made with a microscope—since it completed Harvey's work on the circulation of the blood. Malpighi wrote:

"I see with my own eyes a certain great thing. . . . It is clear to the senses that the blood flowed away along tortuous vessels and was not poured into spaces, but was always contained within tubules, and that its dispersion is due to the multiple winding of the vessels."[33]

The microscopists taken collectively created an epoch in the history of biology, so important is the lens for the advancement of the science.[34] Indeed we find that, broadly speaking, its development along many lines during the eighteenth and particularly the nineteenth century has gone hand in hand with improvements in the compound microscope itself and in microscopical technique. Again, the microscopists in general and Malpighi in particular opened up so many new paths of advance that from this period on it is not possible, even in the most general survey, to discuss the development of biology as a whole. The composite picture must be formed by emphasizing and piecing together various lines of work, such as classification, comparative anatomy, embryology, physiology, heredity, and evolution.

Classification

Classification has as its object that of bringing together things which are alike and separating the unlike. It is "discrimination, description, and illustration—the necessary census task which forms the groundwork on which great theories may be built up"[35]—a problem of no mean proportions when a conservative estimate today shows upward of a million species of animals and plants, leaving out of account the myriads of forms represented only by fossil remains. Naturally the earliest classifications were utilitarian, or more or less physiological: edible and harmful, useful and useless, fish of the sea and beasts of the earth. But as knowledge increased, emphasis was shifted to the anatomical criterion of specific differences and thenceforth classification became at once an important aspect of natural history—a central thread both practical and theoretical. Practical, in that it involved the arranging of living forms so that a working catalog was formed which involved nice anatomical discrimination, and therefore the amassing of a large body of facts concerning animals and plants. Theoretical, because in the process botanists and zoologists were impressed, almost unconsciously at first, with the 'affinity' of various

[33]Foster, op. cit., p. 96.
[34]J. Sachs: History of Botany. English translation, 1890, pp. 220-222.
[35]R. L. Praeger, in Oliver's Makers of British Botany, 1913, p. 220.

types of animals and of plants and so were led to problems of their origin.

From Aristotle who emphasized the grouping of organisms on the basis of structural similarities, we must pass over some seventeen centuries, in which the only work of interest was done by the herbalists and encyclopaedists, to the time of Ray (1628-1705) of Cambridge. As a matter of fact, the Theophrastan classification of plants as Trees, Shrubs and Herbs persisted until the end of the seventeenth century. Previous to Ray the term "species" was used somewhat indefinitely, and his chief contribution was to make the word more concrete by applying it solely to groups of similar individuals, which exhibit constant characters from generation to generation. Covering, as Ray's labors did, the classification of both animals and plants, it is probably not an exaggeration to regard him as the seventeenth century precursor of the great Swedish taxonomist, Linnaeus, for whom he paved the way.[36]

Like many another genius, Linnaeus (1707-1778) was a product of his time and, perhaps, one of the very best examples of the fact that "the most original people are frequently those who are able to borrow the most freely"—to see a great deal in what to others appears commonplace. Linnaeus was first and foremost a botanist. Garnering the best the past had to offer in taxonomy and bringing to bear on it his supreme talent for "classifying, coordinating and subordinating," Linnaeus gave botanical students at once a practical method of classification of flowering plants, based chiefly on the number and arrangement of the stamens. At the same time he insisted on brief descriptions and the scheme of giving each kind of organism a name composed of two words, in which the second word indicates the species and the first, the genus, a group of closely similar species. In short, to name an organism is to classify. Linnaeus' success with botanical taxonomy led him to extend the principles to animals and even to the so-called Mineral Kingdom, the latter showing at a glance his lack of appreciation of any genetic relationship between species.[37]

Indeed the terms genus and species to Linnaeus expressed a transcendental affinity since he believed that species, genera, and even higher groups represented distinct, consecutive thoughts of the Creator. Accordingly, the ultimate goal of taxonomy was to determine the so-called scala naturae. Thus, Linnaeus crystallized two dogmas—constancy and continuity of species—which permeated biology and reached, in slightly different form, their high water mark, indeed a reductio ad absurdum, in Agassiz's Essay on Classification a century later—as fate would have it, just a year before Darwin's Origin of Species.[38]

[36]S. H. Vines: Robert Morrison and John Ray, in Oliver's Makers of British Botany, 1913, p. 9.
[37]J. Sachs, op. cit., p. 108. F. C. Miall: History of Biology, p. 66.
[38]Miall, op. cit., p. 157.

Though today Linnaeus' conception of fixity has been replaced by modifiability of species; the affinity which he recognized and expressed in transcendental terms has given place to similarity based on descent, and his artificial classifications have been superceded by natural classifications, which express, or attempt to express, this genetic connection between species—nevertheless, his greatest works, the Systema Naturae and Species Plantarum created an epoch in biological history, and are by common consent the base line of priority in zoological and botanical nomenclature.[39]

An aftermath of Linnaeus' labors must be mentioned. Naturalists in general and botanists in particular were so captivated with the facility which the Linnean system afforded for cataloging, that collecting and naming became a dominant note for nearly a century. The few who employed microscope and scalpel are outstanding figures on the path to progress.[40]

Comparative Anatomy

The first step toward scientific classification was made, as we have seen, by Aristotle in emphasizing anatomical characters as taxonomic criteria, so that to all intents and purposes classification implies comparison of structural details. Indeed Aristotle recognized the unity of structural plan throughout the chief animal groups, and in reference to man he says "whatever parts a man has before, a quadruped has beneath; those that are behind in man form the quadrupeds back." Not only did he appreciate homology, but also correlation of parts and division of labor in the economy of the animal body.[41] And Theophrastus approached plant morphology in the same philosophical spirit—witness his recognition of the flower as a metamorphosed leafy branch.[42] But it probably would be reading too much into the past to assign the origin of comparative anatomy of animals in the modern sense of the term to Greek, Roman, or early Renaissance science, since description rather than comparison was the key-note. The same may be said of the anatomical work of Vesalius, Harvey, and Malpighi though the latter compared the microscopic structure of various organs, and in his Anatomy of Plants, which shares with Grew's Anatomy the honor of founding vegetable histology, emphasizes the importance of the comparative method. Owing to the less marked structural differentiation of plants in comparison with animals, plant anatomy does not lend itself as readily to descriptive analysis so that an epoch in the study of comparative anatomy is less defined in botany

[39]Systema Naturae, 1735, 10 ed., 1758. Species Plantarum, 1753.

[40]F. W. Oliver: Makers of British Botany, 1913, p. 193. For the point of view of the early part of the nineteenth century, cf. W. Swainson: On the Study of Natural History, 1834.

[41]E. S. Russell: Form and Function; A Contribution to the History of Animal Morphology, 1917, Chapter 1.

[42]Greene, op. cit.

than in the sister science. Therefore both reason and expediency warrant confining our attention to the comparative anatomy of animals.

Probably the first consistent attempt to make a comparative study of the form and arrangements of the parts of animals is represented in a volume published in 1645 by Severinus (1580-1656) of Naples in which he concluded that many vertebrates are constructed on the same plan as man, though Belon, nearly a century earlier, figured and compared the skeletons of bird and man side by side "in the same posture, and as nearly as possible bone for bone."[43] Tyson (1650-1708) of Cambridge at the end of the seventeenth century definitely instituted the monographic treatment of comparative morphological problems in his study of the anatomy of man and monkeys.[44]

Comparative anatomy, however, as a really important aspect of biological work, in fact, as a science in itself, was the result of the life work of Cuvier (1769-1832) of Paris during the first quarter of the last century. It is true that his immediate predecessors, such as John Hunter (1728-1793), the founder of the Hunterian Collection, the nucleus of the anatomical Museum of the Royal College of Surgeons in London, Camper (1722-1789) of Groningen, and Vicq d'Azyr (1748-1794) of Paris, added synthesis to analysis and reached a broader view-point in anatomical study, but Cuvier's claim to fame rests on the remarkable breadth of his investigations—his grasp of the comparative anatomy of the whole series of animal forms.[45] And not content merely with the living, he made himself the first real master of the anatomy of fossil vertebrates and as such is the founder of paleontology.[46]

Cuvier's position in the history of anatomy is largely due to his emphasizing, as Aristotle had done before him, the functional unity of organisms—that the interdependence of organs results from the interdependence of function and that structure and function are two aspects of the living machine which go hand in hand. Cuvier's famous principle of correlation—"Give me a tooth," said he, "and I will construct the whole animal"—is really an outcome of this viewpoint. Every change of function involves a change in structure and therefore, given extensive knowledge of function and of the interdependence of function and structure, it is possible to infer from the form of one organ that of most of the other organs of an animal. "In a word, the form of the tooth implies the form of the condyle; that of the shoulder blade that of the claws, just as the equation of a curve implies all its properties."

[43]F. C. Miall: History of Biology, pp. 18-19.
[44]Cf. Garrison, op. cit., p. 240.
[45]Cf. Russell, op. cit., Chapter 3.
[46]T. H. Huxley: The Rise and Progress of Paleontology, Nature, 24, pp. 452-55, 1881. (Also Coll. Sci. Mem. Vol. 4). O. C. Marsh: History and Methods of Paleontological Discovery, Presidential address, Amer. Assn. Adv. Sci., 1879.

Although Cuvier undoubtedly allowed himself to exaggerate his guiding principle until it exceeded the bounds of facts, he was above all in his science and philosophy a hard-headed conservative and autocrat. He opposed with equal vigor the influence of the Naturphilosophie of Schelling and his school with its transcendental anatomy, Platonic archetypes and the like, as well as the evolutionary speculations of Lamarck and his school. From the vantage points of today we know that in one case he was right and in the other wrong—though in so far as the facts then extended, his opposition was justified in both cases.

Cuvier's immediate successors in France were Milne-Edwards and Lacaze-Duthiers; in Germany, Meckel, Rathke, Müller, and Gegenbaur; in England, Owen and Huxley, and in America, Agassiz, Cope, and Marsh Among these, Owen (1804-1892) perhaps demands special mention. At once a peculiar combination of Cuvierian obstinacy in regard to facts and of transcendental imagination, Owen spent a long life dissecting with untiring patience and skill a remarkable series of animal types, as well as in reconstructing extinct forms from fossil remains. Aside from the facts accumulated, probably his greatest contribution was making concrete the distinction between homologous and analogous structures, which has been of the first importance in working out the pedigrees of plants as well as animals—though Owen himself took an enigmatical position in regard to organic evolution.[47]

Physiology

Anatomy emphasizes the static and physiology the kinetic aspect of the organism, though, as we have seen, structure without function is a lifeless subject and function without structure is an impossibility, since, in Huxley's happy phraseology, physiology is the mechanical, and he would now add, chemical engineering of the living organism.

Animal and plant physiology was discussed by Aristotle, but as might be expected since physiology is more dependent than anatomy upon progress in other branches of science, with less happy results. Similarly, Galen was hampered in his attempt to make physiology a distinct department of learning based on a thorough study of anatomy, and the corner stone of medicine. Like Aristotle he attempted to develop a picture of the modus operandi of the organism, and with such success that fate foisted it upon uncritical generations through fifteen centuries. The worst of it was not that it was nearly all wrong, but that to question Galen's physiology or anatomy was little less than sacrilege until the labors of Vesalius and Harvey brought a realization that Galen had not quite finished the work.[48]

[47]Life of Richard Owen, by his grandson, 1894, vol. 2, pp. 89-96. Also, Essay on Owen's Position in Anatomical Science by T. H. Huxley, in above work.

[48]Foster, op. cit., p. 12.

Neither Vesalius nor Harvey made an attempt to explain the workings of the body by appeal to so-called physical and chemical laws; and for good reason. Chemistry had not yet thrown off the shackles of alchemy and taken its legitimate place among the elect sciences, while during Harvey's lifetime, under the influence of Galileo, the new physics arose. But by the end of the seventeenth century both physics and chemistry, aided by the philosophical systems of Bacon and Descartes, had forced their way into physiology and split it into two schools: the iatro-mechanical founded by Borelli (1608-1679), who by incisive physical methods attacked a long series of problems, frequently with brilliant results; and the iatro-chemical school, which developed from the influence of Franciscus Sylvius (1614-1672) as a teacher rather than as an investigator.[49]

This awakening brought a host of workers into the field and the harvest of the century was garnered and enriched by Haller (1708-1777), the "abyss of learning" of the time, in a comprehensive treatise which at once indicated the erudition and critical judgment of its author and established physiology as a distinct and important branch of biological science, rather than a mere adjunct of medicine.[50] Great as was this contribution of Haller in crystallizing physiology and setting the dividing line between the old and the modern, unfortunately the weight of the author's authority was ranged in favor of two theories which were, in crude form, attracting the attention of biologists—the idea of special vital force and the preformation theory of development.

Perhaps the most significant lines of advance in Haller's century were in setting the physiology of nutrition and respiration—both of which waited upon the work of the chemists—well upon their way toward modern form. Réaumur (1683-1757) of Paris, and Spallanzani (1729-1799) of Pavia may be singled out for their exact studies of gastric digestion which, against the background of the pioneer work during the previous century by van Helmont (1477-1644), Sylvius (1614-1672), Stensen (1638-1686), de Graaf (1641-1673), Peyer 1653-1712), and Brunner (1653-1727), established solution of the food as the main factor in digestion, though it was not clear how these changes differed from ordinary chemical ones. So physiologists of a vitalistic turn of mind cloaked their ignorance under the term "animalization," and left for eighteenth century investigators the establishment of the fact that food in passing along the digestive tract runs the gamut of a series of complex chemical substances, or enzymes, each of which has its part to play in putting the various constituents of the food into such a form that they can pass to the various cells of the body.[51]

[49]Foster, op. cit., Chapters 3 and 6.
[50]A. Haller: Elementa Physiologiae Corporis Humani, 1757.
[51]W. B. Johnson: History of the Progress and Present State of Animal Chemistry, 1803.

On the side of respiration a somewhat closer approach was made toward a true understanding of the process, but there was a better foundation on which to build. The Galenic notion that respiration is a process of refrigeration—a getting rid of the innate heat of the heart and of fuliginous vapors—had been superceded, through the efforts of Harveyan experimentalists—the chemist Boyle (1627-1691), the versatile genius Hooke, the physician Lower (1631-1690), and the lawyer-chemist Mayow (1643-1679). The climax only awaited the overthrow of the Stahlian phlogiston theory, which presented an inverted picture of combustion, and the actual discovery of oxygen. This came in the work of Black, Priestley (1733-1804), Lavoisier (1743-1794), and Girtauner (1760-1800) which made it clear that the chemical changes taking place in respiration involve essentially a process of combustion, and it chiefly remained for later work to show that this takes place in the tissues rather than in the lungs.[52]

Enough perhaps has been said to indicate the trend of physiology away from the maze of Galenic spirits in which science lost itself, toward the modern atmosphere of science with its *working hypothesis*, that life phenomena are an expression of a complex interaction of physico-chemical laws which do not differ fundamentally from the so-called laws operating in the inorganic world, and that the economy of the organism is in accord with the law of the conservation of energy —probably the most far reaching generalization of science during the past century. Although it is difficult to discriminate, certainly the names of Liebig, Wöhler (1800-1882), the brothers Weber, Ludwig (1816-1895), Helmholtz (1821-1894), Müller (1801-1858) and du Bois-Reymond (1818-1896) in Germany; Dumas (1800-1884), Magendie (1783-1855) and Bernard (1813-1877) in France; Donders (1818-1889) in Holland; and Hall (1790-1857) in England were, individually and collectively, chiefly responsible for the reformation of physiology.[53]

Most of the firm foundation on which physiology of animals rests today has been built up by work on vertebrates, though since the middle of the nineteenth century, when the versatile Müller showed the value of studying the physiology of higher and lower animals alike, the science of comparative physiology may be said to have been established.[54] Perhaps it is not an exaggeration to say that the tendency to focus evidence, in so far as possible, from all forms of life on general problems of function represents the present trend of physiological enquiry.

[52]J. Loeb: Dynamics of Living Matter, p. 7. O. Hertwig: The Growth of Biology in the Nineteenth Century, Smithsonian Report, 1900, pp. 461-78.

[53]M. Verworn: General Physiology, English trans., 1899, pp. 16-20. Stirling, op. cit., p. 106.

[54]P. B. Hadley: Johannes Müller, Popular Science Monthly, 1908.

The less obvious structural and functional differentiation of plants retarded progress in plant physiology as it did in plant anatomy. Probably of most historical, and certainly of most general interest is the development of our knowledge of the nutrition of green plants.

Aristotle's notion that the food of plants is prepared for them in the ground was still prevalent at the end of the sixteenth century when Cesalpino, the most philosophic botanist of his day, thought that food enters and passes through vessels and fibers of plants much as oil in a lamp wick, and Jung conceded that plants are not mere passive absorbers of ready-made food, but possess the power of selection from the soil the ingredients needed. Van Helmont (1577-1644), on the border line between alchemist and chemist, who precociously brought to bear the chemical point of view on animal physiology, made the first experiment in plant nutrition on record. He planted a small tree in a large vessel and weighed it. Then after five years, during which time it had only been supplied with water, he found that it had increased some thirty fold in weight and "not suspecting that the plant drew a great part of its materials from the air was forced to exaggerate the virtues of rain-water."[55] Malpighi, however, from his studies on plant histology, gave the first hint of the fact of supreme importance that the crude sap, which enters by the roots, is carried to the leaves where, by the action of sunlight, evaporation, and some sort of a fermentation it is "digested" and then distributed as food to the plant as a whole. But, it is Hale (1677-1761) to whom the botanist looks as the Harvey of plant physiology, for in his Vegetable Statics, published in 1727, he laid the foundations of the physiology of plants by making "plants speak for themselves through his incisive experiments." For the first time it became clear that green plants derive an important element of their food from the atmosphere, and also that the leaves play an active rôle in the movements of fluids up the stem and in eliminating superfluous water through evaporation.[56]

Still the picture was incomplete, and so it remained until the biologist had recourse to further data from the chemist. In 1779 Priestley, the discoverer of oxygen, showed that this gas under certain conditions is liberated by plants. This fact was seized upon by Ingen-Housz (1730-1799) who demonstrated that carbon dioxide from the air is broken down in the leaf during exposure to sunlight, the plant retaining the carbon and returning oxygen—the process of carbon-getting being quite distinct from that of respiration in which carbon dioxide is eliminated. It remained then for de Saussure to show, by quantitative studies of the plant's income, that, in addition to the fixation of carbon, the elements of water are also employed while from the soil various salts including the element nitrogen are obtained. But

[55]Thomson, The Science of Life, p. 70.
[56]F. Darwin: Stephen Hales, in Makers of British Botany, 1913, p. 65.

it was nearly the middle of the last century before the influence of Liebig (1803-1873) and the incisive experiments of Boussingault established the fundamental part played by the chlorophyll of the green leaf in making certain chemical elements available to animals. The realization of the cosmical function of green plants—the link they supply in the circulation of the elements in nature—is a landmark in biological progress, and we may leave the subject here since, except for details in regard to some of the more evident chemical products of photosynthesis and the influence of external factors, the matter still stands essentially where it was in de Saussure's day.[57]

Histology

Studies on the physiology of plants and animals naturally involved the progressive analysis of the physical basis of the phenomena under consideration, but the Aristotelian classification of the materials of the body into unorganized substance, homogenous parts or tissues, and heterogenous parts or organs practically represents the level of analysis until the beginning of the last century. It is true, as we have mentioned, that Hooke in 1665 discovered that cork tissue under the microscope seemed to be composed of little boxes or "cells," and somewhat similar though more extensive observations were made by the contemporary students of the microscope. But another century had nearly elapsed before these microscopic elements were looked at from the point of view of their relation to the development of organisms. Wolff (1733-1794) in 1759 attempted to show the falsity of the prevailing idea that all organisms are preformed in the germ and that the adult state is attained merely by an unfolding and enlarging, by a critical study of the development of animals and plants.[58] And he not only proved his point but also showed that both plants and animals in early developmental stages show a similar fundamental structure, "since every organ is composed at first of a little mass of clear, viscous, nutritive fluid, which possesses no organization, but is at most composed of globules. In this semi-fluid mass cavities are now developed; these, if they remain round or polygonal, become the subsequent cells; if they elongate, the vessels; and the process is identically the same, whether it is examined in the vegetating point of a plant, or in the young budding organs of an animal." But Wolff's refutation of preformation, chiefly through the opposition of Haller, proved abortive, and his observations on cells were so far ahead of the times that they had but slight influence on biological advance. It was not until the revival of interest in plant anatomy early in the last century that the cell became a particular object of study—and still it

[57]H. A. Spoehr: The Development of Conceptions of Photosynthesis since Ingen-Housz, Scientific Monthly, 9, 1919, p. 32.

[58]C. F. Wolf: Theoria Generationis, 1759. W. M. Wheeler: Wolff and the Theoria Generationis, Woods Hole Biol. Lectures, 1898, pp. 265-284.

was the cell wall rather than the contents on which attention was fixed. Then the English botanist Brown discovered the cell nucleus in 1831,[59] quickly followed by the classic investigations of the botanist Schleiden (1804-1881) and the zoologist Schwann (1810-1882), published in 1838 and 1839,[60] which taken together clearly showed that all organisms are composed of units or cells which are at once structural entities and centers of physiological activities. Each cell carries on a double life; one a quite independent and self-contained life; the other a dependent life in so far as the cell has become an integral part of the organism. The life of the organism is the life of the individual cells which compose it. And further, not only are all organisms congeries of cells, but the egg is a cell and the development of animals and plants consists in the multiplication of this initial cell into the multitude of different kinds which constitute the adult: "The elementary parts of all tissues are formed of cells in an analogous, though very diversified manner, so it may be asserted that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells."[61]

Unquestionably the launching of the cell theory represents one of the greatest generalizations in biology, and only needed for its consummation the full realization that the viscid, jelly-like material, which zoologists interpreted as the true living matter of animals, and the quite similar material, which botanists considered the true living part of plants, are practically identical. This conception was grasped in the early sixties by Schultze (1825-1874) in the formulation of the protoplasm theory, and thenceforth not only morphological elements —cells—but also the material of which they are composed—protoplasm —was recognized as fundamentally the same in all living beings. Indeed, the realization of a common physical basis of life in both plants and animals—a common denominator to which all vital phenomena are reducible—gave content to the term biology and created the science of life in its modern form.[62]

Embryology

The enunciation of the cell theory came, as we have seen, from combined studies on the adult structure and on the development of plants and animals from the germ or egg, and accordingly implies that the science of embryology has a history of its own. As a matter of fact, Aristotle discussed the wonder of the beating heart in the hen's egg after three days' incubation, but there the subject practically rested

[59] J. B. Farmer: Robert Brown, in Makers of British Botany, 1913, p. 119.

[60] M. J. Schleiden: Ueber Phytogenesis, 1838. T. Schwann: Mikroscopische Untersuchungen ueber die Uebereinstimmung inder Structur und dem Wachstum der Thiere und Pflanzen. English translations by H. Smith, 1847.

[61] Schwann, op. cit., English trans. p. 165.

[62] E. B. Wilson: The Cell in Development and Inheritance, 2d ed., 1900. Verworn, op. cit.

until Fabricius (1537-1619), early in the seventeenth century, published a treatise which illustrated the obvious sequences of events within the hen's egg to the time of hatching.[63] This beginning was built upon by a pupil of Fabricius, the celebrated Harvey, who added many details of interest and insisted, as Aristotle had before him, that the embryo arises as a gradual differentiation of unformed material of the egg.[64]

However, little progress in embryology was possible without the microscope which was first applied to the problem by the versatile Malpighi, in two treatises sent to the Royal Society in 1672.[65] One has but to study his splendid series of illustrations to realize how animal development was placed upon a plane so advanced that for over a century it was unappreciated. One conclusion of Malpighi, however, was seized upon by contemporary biologists. Apparently, unbeknown to him, some of the eggs which he studied were slightly incubated so that he thought traces of the future organism are preformed in the egg. This error, coupled, for example, with Swammerdam's observation of the fact that parts of the adult insect are delineated in the larva ready to pupate, crystallized the preformation theory which denied all true development or epigenesis, as advocated by Aristotle and Harvey, and held that the future adult characters pre-exist in miniature in the egg. Even the acute observations of Wolff in his embryological classic, to which we have referred, failed of fruition since it negated the preformation idea which, in the years that had elapsed since Malpighi, had become the dominant question in embryology. Indeed the theory was carried to a reductio ad absurdum by Haller, Bonnet (1720-1793) and others who accepted the logical conclusion that:

> Each seed includes a plant: that plant, again,
> Has other seeds, which other plants contain:
> Those other plants have all their seeds; and those,
> More plants, again, successively inclose.
> * * * * * * * * *
> So Adam's loins contain'd his large posterity,
> All people that have been, and all that e'er shall be.
>
> Amazing thought! what mortal can conceive
> Such wond'rous smallness! Yet we must believe
> What reason tells: for reason's piercing eye
> Discerns those truths our senses can't descry.

So Baker expressed it in one of the few departures from prose permitted in the Philosophical Transactions of the Royal Society.[66]

The truth of the matter is that the time was not ripe for theories of development. The preformationists were wrong but so were Aristotle, Harvey, and Wolff who went to the other extreme and denied all egg

[63]Locy, op. cit., p. 43. Russell, op. cit., p. 113.
[64]W. Harvey: Exercitationes de Generatione Animalium, 1651.
[65]Locy, op. cit., p. 202.
[66]H. Baker: The Microscope Made Easy, 2d ed., 1743, p. 252.

organization and therefore tried to get something out of nothing. It remained for the present generation of embryologists to work out many of the details of the origin of the germ cells and their organization, and to reach a level of analysis deep enough to suggest how "the whole future organism is potentially and materially implicit in the fertilized egg cell," and thus that "the preformationist doctrine had a well concealed kernel of truth within its thick husk of error."[67]

The real step to progress, Baker's implicit confidence in "reason" to the contrary, came in the accurate and comprehensive studies of von Baer (1792-1876) published in the thirties of the last century.[68] Taking his material from all the chief groups of higher animals von Baer founded comparative embryology. Among his achievements may be mentioned the clear discrimination of the chief developmental stages, as cleavage of the egg, germ layer formation, tissue and organ differentiation; the importance of the facts of development for classification, and the discovery of the egg of mammals. His observations on the origin and development of the germ layers, which afforded the key to many general problems of morphogenesis, and his emphasis on the resemblance between certain embryonic stages of higher animals and the adult stages of lower forms, were exaggerated and crystallized by his successors, under the influence of the evolution theory, as the germ layer theory and the recapitulation theory, or von Baer's law. Both were of the greatest importance in stimulating research for half a century—and if the present generation has not inherited its forebears' implicit faith in the theories, it at least has profited immensely by the facts they accumulated.[69]

From every point of view von Baer created an epoch in embryology synchronous with the formulation of the cell theory by Schleiden and Schwann, and it thenceforth became the problem of the embryologist to interpret development in terms of the cell. Time will not permit us to follow the establishment of the fact that the egg and the sperm are really single nucleated cells; that fertilization consists in the fusion of egg and sperm and the orderly arrangement of their chief nuclear contents, or chromosomes; that the new generation is the fertilized egg since every cell of its body as well as every chromosome in every cell is a lineal descendant by division from the egg, and so from the germ cells which united at fertilization to form it. Such, however, are the chief results of cytological study since von Baer; but embryologists have not been content to employ merely the descriptive method, and the

[67]Thomson, op. cit. C. O. Whitman: Woods Hole Biological Lectures, 1894, pp. 205-272. E. B. Wilson: The Problem of Development, Science, 21, 1905, pp. 281-294.

[68]K. E. von Baer: Ueber Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion, 1828-37. Cf. Huxley: Philosophical Zoology Selected from the Works of K. E. von Baer, in Scientific Memoirs, February and May, 1853.

[69]Locy, op. cit., pp. 214-222.

dominant note of the most modern research under the influence of Roux is physiological—the experimental study of the significance of fertilization, the dynamics of cell division, the basis of differentiation, the effect of environmental stimuli, and so on.[70]

Genetics

It is but natural that the study of inheritance could be little more than a groping in the dark until embryology, under the influence of the cell theory, afforded a body of facts which clearly indicated that the fertilized egg is typically the sole bridge of continuity between successive generations. Indeed the present science of genetics has a history confined solely to post-Darwinian times and mostly to this century.

Although clearly suggested by a number of workers, the conception of the continuity of the germ cells—or germ plasm—was first forced upon the attention of biologists and given greater precision by Weismann (1834-1914) in a series of essays culminating in 1892 in his volume entitled The Germ Plasm. He identified the chromatin material which constitutes the chromosomes of the cell nucleus as the specific bearer of hereditary characters, and emphasized a sharp distinction between the cellular derivatives of the fertilized egg—on the one hand, the somatic cells which by division and differentiations build up the body of a higher plant or animal; and on the other, the germ cells which are destined to play but little part in the life of the individual which bears them, but instead are to be liberated and give rise to the next generation. The importance of this distinction can hardly be over emphasized for at once it makes clear that, for all practical purposes, the bodily characteristics of an individual are negligible from the stand point of heredity, since the offspring are descendants not from the body cells, but from the germ cells which it carries—and these in turn from the germ cells of the preceding generation. As Weismann insisted, this view makes it difficult to conceive how modifications of the soma can so specifically affect the germ cells which it bears that the latter can reproduce the modifications—in other words that so-called "acquired characters" can not be inherited. And there is no satisfactory evidence that such characters are inherited. The practical bearings of this conclusion are obviously of the highest importance, lying as they do at the very root of many questions in regard to the factors of evolution, not to mention such practical ones as education and eugenics.

While this viewpoint has been gradually gaining content and precision, the science of heredity has been advancing not only by exact studies of the structure and physiology of the germ cells, but also by statistical investigations of the results of heredity—the various characters of animals and plants in parent and offspring.

[70]Wilson, op. cit.

The first studies of this type which attracted the attention of biologists were made by Galton (1822-1911), who in the eighties and nineties of the last century amassed a large amount of data in regard, for example, to the stature of children with reference to that of their parents, and formulated his well known "laws" of inheritance.[71] But the epoching making work which eventually created the science of genetics was that of an Austrian monk, Gregor Mendel (1822-1884), who combined in a masterly manner the experimental breeding of pedigreed strains of plants and the statistical treatment of the data thus secured in regard to the inheritance of sharply contrasting characters, such as the flower color in sweet peas. Mendel's work was published in 1865 in an obscure natural history periodical[72] and he himself abandoned his teaching and research to become the Abbot of his monastery. Thus terminated prematurely the productive work of one of the epoch makers of biology, and the now famous "Mendelian laws" of inheritance were unknown to science until 1900 when other biologists, coming to similar results, unearthed his forty-year-old paper. We can pause only to say that the fundamental principle of the segregation of the genes of the alternative characters within the germ cells, which Mendel's work indicated, has been extended to other plants and to animals, and from being, as at first thought, a principle of rather limited application, now seems to be the key to all inheritance. And the present results are extremely convincing because cytological studies on the architecture of the chromosome-complex of the germ cells keep pace and afford a picture of the physical basis—of the mechanism by which the segregation and distribution of characters by the Mendelian formula takes place.[73] Such is the deeply hidden germ of truth in the old preformation theories!

Origin of Life

With our present conception of the complexities of organisms it is difficult to realize that up to the seventeenth century naturalist and layman saw nothing more incongruous in the spontaneous origin of nearly all kinds of plants and animals than does the boy of today who believes that horse hairs soaked in water are transformed into worms. Even Aristotle thought that certain of the vertebrates, such as eels, arose spontaneously, and Harvey accepted the same view of the origin of many forms of life. It remained for Redi (1626-1698) to lay aside discussion for experiment. By protecting decaying meat from contamination by flies he demonstrated that these insects are not developed from the flesh and that the apparent transformation of meat into maggots

[71]F. Galton: Natural Inheritance, 1889.

[72]G. J. Mendel: Versuche über Pflanzen-Hybriden, Verhandlungen des naturforschenden Vereines in Brünn, Bd. 4, 1865.

[73]T. H. Morgan: The Physical Basis of Heredity, 1919.

is due solely to the eggs of flies being deposited thereon.[74] But the time-honored doctrine was not overthrown by this experiment or the long series which Redi made, for the presence of parasites within certain recondite parts of higher animals baffled Redi himself, while improvements in the microscope soon revealed a microcosm whose origin seemed plausibly explained as spontaneous. Biogenesis, or all life from preexisting life, was placed on a secure foundation only within the past sixty years by the working out of the remarkably complex life histories of internal parasites and by the classical demonstrations of Pasteur and others that micro-organism are not the result but the cause of decay; a fact which is at the basis of and is attested by the methods now universally in use in food preservation and aseptic surgery—to mention but two instances.[75] The vicissitudes of the doctrine of biogenesis—" la génération spontanée est une chimère," wrote Pasteur—is an eloquent illustration of the aphorism of the old London microscopist that "the likeliest method of discovering truth is by the observations and experiments of many upon the same subject."[76]

Organic Evolution

Since we have every reason to believe that all life now arises from preexisting life and has done so since matter first assumed the living state, it apparently follows that the stream of life is continuous from the remote geological past to the present and that all organisms of today have an ancient pedigree. This leads us to a question which has interested and perplexed thinking men of all times, how things came to be as they are today? It was the Greek natural philosophers who projected the idea of history into science and attempted to substitute a naturalistic explanation of the Earth and its inhabitants for the established theogenies, and thus started the uniformitarian trend of thought which culminated in the establishment of organic evolution during the past century.

Again it is Aristotle who is singled out among the Greeks for his combination of sound philosophy and induction which reaches no higher expression than in his statements regarding the relationships of organisms. He says, in substance: Although the line of demarcation is broadly defined, yet nature passes by ascending steps from one to the other. The first step is that of plants; which, compared with animals, seem inanimate. The second step nature takes is from plants to plant-animals, the zoophytes. The third step is the development of animals, which arise from an increased activity of the vital principle,

[74]F. Redi: Esperienze Intorno alla Generazione Degl'Insetti, 1668. English trans. 1909.

[75]T. H. Huxley: Biogenesis and Abiogenesis. Presidential Address, British Assn. Adv. Sci., 1870. Collected Essays, Vol. 8, L. L. Woodruff: The Origin of Life, in the Evolution of the Earth and Its Inhabitants, R. S. Lull, editor, 1918.

[76]Baker, op. cit., p. V.

resulting in sensibility; and with sensibility, desire; and with desire, locomotion. Man is the head of animal creation. To him belongs the God-like nature. He is preeminent by thought and volition. But although all are dwarf-like and incomplete in comparison with man, he is only the highest point of one continuous ascent.[77]

Broadly speaking, Aristotle apparently held substantially the modern idea of the evolution of life from a primordial mass of living matter to the highest forms, and believed that evolution is still going on—the highest has not yet been attained. In looking for the effective cause of evolution Aristotle rejected Empedocles' hypothesis of the chance play of forces, which embodied in crude form the idea of the survival of the fittest, and substituted secondary natural laws to account for the fact that "Nature produces those things which, being continually moved by a certain principle contained in themselves, arrive at a certain end." As Osborn points out, Aristotle's rejection of the hypothesis of the survival of the fittest to account for adaptations of organisms was a sound induction from his necessarily limited knowledge of nature—but had he accepted it he would have been "the literal prophet of Darwinism."[78]

Although the thread of continuity of evolutionary thought is not broken from Aristotle to the present, no historical interest will be served in following the poetical expression by Lucretius, the discussion at once broad and narrow of the most liberal medieval Churchmen, the "Arab philosophy—a system of Greek thought expressed in a Semitic tongue and modified by Oriental influences," or the vagaries of the Renaissance naturalists and speculative evolutionists, who, with a minimum of fact and a plethora of imagination were the worst enemies of the evolution idea. In truth, the great natural philosophers from Bacon and Leibnitz to Kant and Hegel laid the broad foundation for our modern attack on evolution, but from the strictly biological viewpoint, two Frenchmen, Buffon and Lamarck, and two Englishmen, Erasmus Darwin and his grandson, Charles Darwin, stand preeminent, and the greatest is Charles Darwin.

Buffon (1707-1778) was a peculiarly happy combination of parlor entertainer and scientist—entertaining by each new volume of his great Natural History the social set of Paris, and instructing them at the same time. And it was largely between the lines of his Natural History that Buffon's evolutionary ideas found expression; but expressed they are, though sometimes difficult to decipher—beyond the ken, Buffon hoped, of the censor and dilettant, for apparently he was not of martyr stuff.[79] It is not strange, therefore, that there are some differences of opinion amongst biologists today as to just how much

[77]Lewes, op. cit., pp. 189-96. Osborn, op. cit., p. 48.
[78]Osborn, op. cit., pp. 55-57.
[79]S. Butler: Evolution Old and New. 3d Edition, 1911, p. 78. A. O. Lovejoy: Buffon and the Problem of Species, Pop. Sci. Monthly, 1911.

weight is to be placed on some of Buffon's statements, but certainly it is not exaggerating to ascribe to him not only the recognition of the factors of geographical isolation, struggle for existence, artificial and natural selection in the origin of species, but also, which is equally important, the propounding of a theory of the origin of variations. He thought that the direct action of the environment brings about modifications of the structure of animals and plants and these are transmitted to the offspring.[80]

When Buffon's influence was at its zenith, Erasmus Darwin (1731-1802), a successful medical practitioner, expressed consistent views on the evolution of organisms in several volumes of prose and poetry.[81] Although a contemporary critic in the Edinburgh Review remarked that Darwin's "reveries in science have probably no other chance of being saved from oblivion, but by having been married to immortal verse," today biologists recognize him as the anticipator of Lamarck's doctrine that variations spring from within the organism through its reaction to environmental conditions. "All animals undergo perpetual transformations which are in part produced by their own exertions, in consequence of their desires and aversions, of their pleasures and their pains, or of irritations, or of associations; and many of these acquired forms or propensities are transmitted to their posterity."[82]

While Cuvier was extending and synthesizing the knowledge of anatomy of living and extinct forms and founding the so-called school of facts, his fellow-countryman Lamarck (1744-1829), on the basis of work first on plants and then on animals, carried on in fearless manner the evolutionary inspiration of Buffon and Erasmus Darwin (though the latter's works may not have been known to him), and established the coterie of evolutionists in Paris each of whose essays Cuvier hailed as a "new folly." Lamarck developed with great care the first complete and logical theory of organic evolution, and is the one outstanding figure in biological uniformitarian thought between Aristotle and Charles Darwin. "For nature," he writes, "time is nothing. It is never a difficulty, she always has it at her disposal; and it is for her the means by which she has accomplished the greatest as well as the least of her results. For all the evolution of the earth and of living beings, nature needs but three elements—space, time, and matter."[83]

In regard to the factors of evolution, Lamarck emphasized the indirect action of the environment in the case of animals, and the direct action in the case of plants. The former are induced to react and thus

[80]Thomson, op. cit., pp. 219-220.

[81]Botanic Garden, 1791, Zoonomia, 1794-96; Phytologia, 1800; Temple of Nature, 1802. It is said that Paley's famous "Natural Theology" was written to counteract the influence of the Zoonomia.

[82]Zoonomia, 1st ed., p. 503. Cf. E. Krause: Life of Erasmus Darwin, with a Preliminary Notice by Charles Darwin. 1879.

[83]Hydrogéologie, 1802.

adapt themselves, while the latter, without a nervous system, are moulded directly by their surroundings. And, so Lamarck believed, such bodily modifications—acquired characters—are transmitted to the next generation and bring about the evolution of organisms.[84]

Through the influence of Cuvier, and the relative weakness of Lamarck's successors—the foremost was Etienne Geoffroy-Saint-Hilaire (1772-1844)[85]—the French School of evolutionists dwindled to practical extinction, while in Germany, Goethe (1749-1832), the greatest poet of evolution, and Treviranus (1776-1837) "brilliantly carried the argument without carrying conviction," for the man and the moment must agree. Then in England the uniformitarian ideas of Hutton (1726-1797), elaborated by Lyell (1797-1875) in his "Principles of Geology, being an attempt to explain the former changes of the Earth's surface by reference to causes now in action" (1830-1833), created an epoch in geology. The prevailing doctrine of cataclysms, emphasized among biologists especially by Cuvier, gradually gave place to that of uniformity—an orderly evolution of the Earth—and paved the way for the next logical step—the evolution of the Earth's inhabitants.[86]

It has been truly said that the idea of development saturated the intellectual atmosphere. But intrenched prejudices which hampered the acceptance of evolution in the inorganic world were immeasurably augmented when the world of life was approached, and only an overwhelming amount of scientific evidence, impartially and convincingly presented could carry conviction.[87] This, in part, accounts for the slight influence of the work of the earlier evolutionists, as well as for the reception accorded the evolutionary views expressed anonymously in the Vestiges of the Natural History of Creation[88] by Chambers (1802-1871). The ten editions of this work (1844-1860) created a furor, especially in England, and were opposed alike by biologist and layman.

The case for evolution at the time, as it appeared to the erudite Whewell, is thus summarized in the first edition (1838) of his well known "History of the Inductive Sciences" and reiterated in the third edition (1857): "Not only is the doctrine of the transmutation of species in itself disproved by the best physiological reasonings, but the additional assumptions which are requisite, to enable its advocates to apply it to the explanation of the geological and other phenomena of the earth, are altogether gratuitous and fantastical. Such is the

[84]Philosophie Zoologique, 1809. English trans. by H. Elliot, 1914. Osborn, op. cit., pp. 165-167. A. S. Packard: Lamarck, the Founder of Evolution, 1901.

[85]Philosophie Anatomique, 1818.

[86]J. W. Judd: The Coming of Evolution, 1910.

[87]Cf. A. O. Lovejoy: The Argument for Organic Evolution before "The Origin of Species." Pop. Sci. Monthly, 75, 1909.

[88]A. Ireland: Introduction to the 12th Edition of the "Vestiges," 1884.

judgment to which we are led by the examination of the discussions which have taken place on the subject."[89]

And then appeared the greatest work of Charles Darwin (1809-1882)—the result of twenty years' labor. The Origin of Species (1859) presented a huge amount of data which most reasonably could be explained by assuming the origin of existing species by descent with modifications from others, and also offered as the modus operandi of their origin the theory of "natural selection, or the preservation of favored races in the struggle for life." In Darwin's words: "As many more individuals of each species are born than can possibly survive, and as, consequently, there is frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable to itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving, and thus be naturally selected. From the strong principle of inheritance any selected variety will tend to propagate its new and modified form."

Facts and theories had been brought forward before in support of evolution—indeed the theory of natural selection had been suggested before Darwin's time and again independently by Wallace (1822-1913) just as Darwin was completing his long studies preparatory to publication.[90] But the stupendous task of thinking evolution through for the endless realm of living nature remained to be done, and Darwin did it convincingly by his brilliant, scholarly, open-minded, and cautious marshalling and interpreting of data.[91]

It was the combination of the facts and the theory to account for the facts which won the thinking world to organic evolution and "made the old idea current intellectual coin." Darwin supplied the Ariadne thread which led from the maze of transcendental affinity to genetic continuity. Now we know that evolution is a bird's-eye view of the results of heredity since the origin of life and that the facts of inheritance hold the key to the factors of evolution.

Darwin spent the twenty years subsequent to the publication of the Origin of Species, as he had spent the preceding twenty years, in study and research, the results of which appeared in nine additional volumes. Three of these perhaps may be singled out as primarily an elaboration of the "Origin": The Variation of Animals and Plants under Domestication (1868), The Descent of Man (1871) and The Expression of the Emotions (1872). Singly and collectively these volumes are a monu-

[89] 3d Ed., Vol. 3, p. 481.

[90] A. R. Wallace: The Origin of the Theory of Natural Selection. Reply on receiving the Darwin-Wallace medal of the Linnean Society, July, 1908. Pop. Sci. Monthly, Apr. 1909, p. 396. J. Hooker: The First Presentation of the Theory of Natural Selection. Op. cit., p. 402.

[91] Cf. the Darwin Centennial Number of the Pop. Sci. Monthly, April, 1909.

ment to genius and labor. Erasmus Darwin was wont to say that the world is not governed by brilliancy but by energy. His grandson revolutionized biological thought through their combination.

Among Darwin's early converts from the ranks of professional biologists must be mentioned Huxley (1825-1895)[92] and Hooker (1817-1911) in England, Haeckel (1834-1919) and Weismann in Germany, and Gray (1810-1888) in America—men with the courage of their convictions when courage was necessary, whose support did so much for the promulgation of evolutionary ideas.

> "Thoughts that great hearts once broke for, we
> Breathe cheaply in the common air."

Today no representative biologist questions the fact of evolution—"evolution knows only one heresy, the denial of continuity"—though in regard to the factors there is much difference of opinion. It may well be that we shall have reason to depart widely from Darwin's interpretation of the effective principles at work in the origin of species, but withal this will have little influence on his position in the history of biology. The great value which he placed upon facts was exceeded only by his demonstration that this "value is due to their power of guiding the mind to a further discovery of principles." Darwin brought biology into line with the other inductive sciences, recast practically all of its problems, and instituted new ones.

Such, in briefest form, is a survey of the epochs and epoch-makers in biological progress—a mere glance of the biologist into the past "to the mountains whence cometh his strength." Building upon these foundations the biological sciences are developing with amazing rapidity at the present time chiefly through the cumulative influence of an all pervading desire of students of life phenomena to observe nature at work—actually to control and modify biological processes. Today the investigator insists upon interrogating nature experimentally and observing the modus operandi. In a word, the modern biological ideal is to construct an account of the living organism which can be verified by actual observation provided the proper conditions are afforded. Biology has emerged from the phase of development in which the descriptive note was dominant and has become in fact an experimental science.

[92]T. H. Huxley: Darwiniana, Collected Essays, Vol. 2. Life and Letters, edited by his son, 1901.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1921, xviii, pp. 183–184.

The food reactions of the infusorian Spathidium spathula.

By LORANDE LOSS WOODRUFF and HOPE SPENCER.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Spathidium spathula is an holotrichous infusorian whose sole diet consists of smaller ciliates, chiefly species of *Colpidium*. If the truncated anterior end of a *Spathidium*, which is swimming forward and revolving on its long axis, happens to come into direct contact with a *Colpidium*, the latter usually becomes motionless at once and its protoplasm shows signs of pathological changes.

Paralysis and death of the prey is apparently brought about by the liberation of a specific substance from the oral region of *Spathidium* which is toxic to small ciliates. Various authors have ascribed this result to trichocysts about the peristome, but as a matter of fact none are present. However, a number of rod-like bodies can be demonstrated in the thickened rim of the peristome which may represent the seat of the poison. One is at liberty to interpret these bodies as "trichocyst material," though the poisonous nature of trichocysts, even in *Paramecium*, remains to be demonstrated.

The paralysis of a *Colpidium* by a *Spathidium* results in a marked change in the latter's behavior, which up to this instant has comprised random swimming movements. If the *Colpidium* happens to remain against the truncated anterior end, the *Spathidium* forthwith proceeds by means of the thickened edges of the peristome to force its prey down through the slit-like mouth into the interior of the cell. On the other hand, if the *Colpidium* is not instantly rendered motionless and becomes removed a short distance from the oral region of its captor, the latter institutes a series of rapidly repeated avoiding reactions. This behavior has a tendency to keep the animal in the general vicinity of its quarry because each time the Spathidium starts away its forward progress is checked by the reaction. It is obvious that sooner or later, merely on the basis of chance, the anterior end of the animal will

again come in contact with the *Colpidium*, but a careful study of many captures shows beyond doubt that the apparently random reactions are successively modified so that the peristome of *Spathidium* is brought gradually nearer and nearer until it grasps the *Colpidium*. In brief, *Spathidium* in the majority of cases recovers its lost prey, even though the latter may have become removed a distance equal to several times the length of the *Spathidium*.

The factors involved here may be products derived from the dead *Colpidium*, but more probably are directly related to the poisonous secretion liberated by *Spathidium* itself. In any event, the complex series of reactions exhibited by *Spathidium* afford, it is believed, a remarkable example of sensing at a distance by a unicellular organism.

The complete paper will appear in the Journal of Experimental Zoology.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, MARCH 14

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 32, No. 3, April, 1921

EXPERIMENTS ON THE EFFECT OF REMOVAL OF THE PRONEPHROS OF AMBLYSTOMA PUNCTATUM[1]

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TWENTY-THREE FIGURES

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INTRODUCTION

The common occurrence, among the lower orders of vertebrates, of a more or less persistent head kidney or pronephros has led to the accumulation of a very comprehensive literature on this subject from the standpoint of pure morphology. Little evidence has been furnished in any group, however, of the rôle which these organs play in the life of the embryo.

Price ('10), working on the head kidney of one of the myxinoids, Bdellostoma stouti, a form in which it persists throughout adult life, follows up his earlier descriptions of the development

[1] A preliminary report of the results obtained was published in 1916. (Howland, R. B., '16. On the Effect of Removal of the Pronephros of the Amphibian Embryo. Proc. Nat. Acad. Sc., vol. 2.)

of the excretory system with a study of both the structure and the function of this organ in its fully formed state. The head kidney of Bdellostoma is shown to be a composite structure, possessing at its earliest appearance all the characteristics of a pronephros, with the single exception of the typical glomerulus, but later fusing with the anterior end of the mesonephros and losing all connection with the exterior. Structurally, then, the head kidney in at least one of the myxinoids is rendered incapable of playing the rôle of an excretory organ, but since it is connected with both coelomic cavity and the circulatory system, and since, also, it has been proved possible to transfer certain substances from the coelomic fluid directly into the circulation, Price concludes that its probable function is the transference of lymph from the body cavity into the blood-vessels.

Since the discovery of the organ by Johannes Müller ('29), the origin, development and morphology of the amphibian pronephros have been described by many investigators, chief among whom are von Wittich ('52), Fürbringer ('78 a), and Field ('91). An excellent review of the early controversies concerning its structure is also given by Field. The presence of a well-developed pronephros in the amphibian embryo, its early appearance, and its relatively large size have led to the general assumption that it is a functioning organ. Its characteristic structure, consisting in a glomerulus which extends freely into the coelomic cavity, a coiled tubule furnished with open ciliated funnels for the intake of coelomic fluid, and a simple duct establishing direct connection with the exterior, further points to its function as excretory in nature. Still, from the physiological viewpoint, no experimental evidence as to the extent to which the embryo is dependent upon it for the elimination of excretory products had been offered at the time of publication of my first note ('16); Since then Swingle (19), working independently upon the embryo of Rana sylvatica, has obtained results which in the main agree with my own.

In the series of experiments described in the present paper, the necessity of these organs for the life of the embryo has been proved by the fact that death follows, in time, after the removal

of both pronephroi. The uniform occurrence of a pronounced edema after bilateral extirpation, similar to the condition which follows certain pathologic conditions in the permanent kidneys of the higher animals, suggests a further parallel between the larval kidneys on the one hand and the permanent kidneys on the other with respect to their function. Extirpation of the coiled portion of one or both pronephroi has also afforded the opportunity of investigating the question of correlation in development through a study of the effect of its removal on the other components of the excretory system. The response of one kidney in cases where it has been left functioning alone has further led to the consideration of the factors involved in the restoration of the normal secreting area through the process of compensatory hypertrophy.

Although no invariable rule can be formulated as to the type of regulation which may be anticipated as a consequence of the abnormal conditions imposed by extirpation of an embryonic region or organ, a survey of the results obtained in the many instances already investigated shows that, in a large proportion of cases, there occurs a more or less complete regeneration of the excised part. Byrnes ('98 b) and Harrison ('18) have shown, for instance, that the limbs of the amphibian embryo, if removed at an early age, will soon be replaced through the regenerative capacity of the surrounding tissue. This is also true of the auditory and nasal placodes, the lens, and the gills. The possession by the amphibia, of the regenerative power to such a high degree would naturally lead to the presumption that removal of the pronephric rudiment might result in a similar replacement of this organ. This, however, as will be shown later, is not the case in the Amblystoma larva, for the adjustment consequent on removal of the pronephros is not in the nature of a restitution, but is a compensating hypertrophy[2] of the remaining head kid-

[2] A peculiar instance of compensatory hypertrophy in another organ is cited by Kochs ('97) in his work on Triton, where the amputation of the fore leg often resulted in a marked hypertrophy of the tail. Retardation or acceleration in the growth of a fore leg in the larvae of Rana esculenta and Bufo viridis may be induced by removal of a hind leg, according to Kammerer ('05), the hastening or arrest of growth depending on the rapidity of wound healing.

ney and of its duct—such compensation as is common in the adult kidney of the higher vertebrates. The degree of compensation which has been attained by the single kidney has been estimated in terms of increase in the secreting surface of the pronephric tubules, as well as by measurement of the volume of the cells making up the walls.

Although the literature on the subject of hypertrophy in the kidney of the higher forms is too extensive to permit of a discussion in any detail in the present paper, it may be well to mention several of the more important standpoints from which the subject has been treated.

The question of direct causal connection between the demand for increased functional activity and the changes in the compensating organ has been definitely settled by such experimental studies as those of Sacerdotti ('96), in which the kidneys of unoperated dogs were stimulated to compensatory overgrowth by injection of the blood from completely nephrectomized animals. In this instance, as in all the early pathological literature dealing with this subject, the exact nature of the histological changes evoked in the stimulated organ was given only secondary consideration. Recently, however, with the general acceptance of the distinction between the terms hypertrophy and hyperplasia, more accurate observations of the condition of the kidney constituents consequent on increased activity have been made. Both forms of regulation may occur in the same organ, each being limited to a definite area. Wolff ('00), in his contributions on the macroscopic and microscopic conditions of the hypertrophied kidney after resection, draws a sharp distinction between those changes which occur in the region of the lesion, and those occurring in the uninjured portions of a resectioned kidney. In the former location he observes that mitoses appear at the end of two days, resulting in the formation of new epithelium. In the uninjured portion of the remaining kidney tissue, however, no new formation of either urinary tubules or of glomeruli takes place as compensation for those excised, but here a sufficient restitution occurs through increase in size, and the normal balance is restored. From the histological viewpoint,

this process is sure to be almost entirely one of hypertrophy, not a hyperplasia, of the kidney elements. In the glomerulus and urinary tubules the former process occurs exclusively, in the epithelial cells themselves, hypertrophy with a negligible amount of hyperplasia.

The clearest and by far the most accurate statement of the exact histological conditions found in the hypertrophied kidney is given, however, by Galeotti and Villa Santa ('02). These authors approached the problem from a widely different viewpoint, their main object being to determine whether the hypertrophied kidney of an adult animal would show the same histogenetic potency as that of an animal which had not attained its full growth. From their study of the kidneys of young and adult dogs and rabbits, careful estimates were made of the number of glomeruli found in sections of normal and hypertrophied kidneys and of the relationship between the average surface area of the glomeruli and the number present. Furthermore, the diameter of the lumen in the tubuli recti was accurately measured and the secreting surfaces in the two kidneys obtained for comparison. The volumes of the cell walls in the two cases were also computed and contrasted. The thoroughness of the methods used in these computations gives added weight to their conclusion that, whereas in the young kidney hyperplasia may occur, the adult kidney has lost its potency for addition of new parts, and can only respond by the enlargement of those elements already present.

Kittleson ('20), in his recent report on the effects of inanition and refeeding on the growth of the kidneys in young rats, confirms the opinion of former workers that starvation inhibits the formation of renal corpuscles, and further concludes that "refeeding after stunting results not only in a hypertrophy of the renal corpuscles but also in an increase in number, which may even exceed the normal." This would indicate the possibility of both hypertrophy and hyperplasia of the same kidney element, induced by these abnormal conditions.

No instances are on record of experiments dealing with the production of hypertrophy or hyperplasia in the adult am-

phibian kidney, although Levi ('05) claims that the anuran mesonephros has, in one species, the power of regeneration after injury. He destroyed both the urogenital anlage and that of the wolffian duct and tubules in Bufo larvae by means of a red-hot needle, and obtained after a certain time complete regeneration of the excretory organs. His method is, however, open to the criticism that no accurate estimate of the actual extent of the injury done to the organs by this type of operation can be made. The introduction of a hot needle may cause only minor displacement or destruction of a few cells of the duct or coils. Certainty as to the degree of injury can be assured only by complete excision.

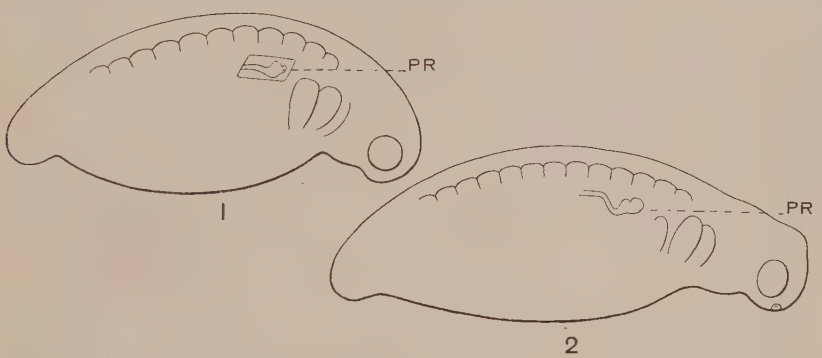
Removal of a portion of a given organ or system has been found to have a varied effect on the formation of its other constituents. The extent to which the growth of one part is influenced during its development by other developing portions of the embryo varies widely, the scale of difference ranging from complete interdependence to those extremes in which each constituent possesses the potency for self-differentiation without the influence of any formative stimuli.

In view of this variation in the degree of correlation exhibited by closely related parts during their early growth, it was of interest to consider, particularly in cases of bilateral extirpation of the pronephros, the effect of the absence of the tubules on the formation of the glomeruli. The operated animals showed without exception a differentiation of glomerular tissue perfectly normal in position and size. The glomerulus therefore possesses the power of self-differentiation, and is entirely independent of the presence of the tubular elements.

It gives me great pleasure to acknowledge my indebtedness to Prof. R. G. Harrison, at whose suggestion this investigation was begun, for his helpful and constructive criticism during the course of my work.

MATERIAL, METHODS AND NORMAL DEVELOPMENT

Embryos of Amblystoma punctatum were used for all the experiments. The stages chosen for operation varied from the condition in which the first loop of the pronephric tubules appears as a slight, ventrally directed curve of the duct (fig. 1, stage 30)[3] to that in which the two funnels, together with the first loop, appear as a broadened Y (fig. 2, stage 32). In all cases embryos were used before contraction of the body muscles began, as movement not only hindered the operation, but often tore open the wound after successful removal of a kidney.



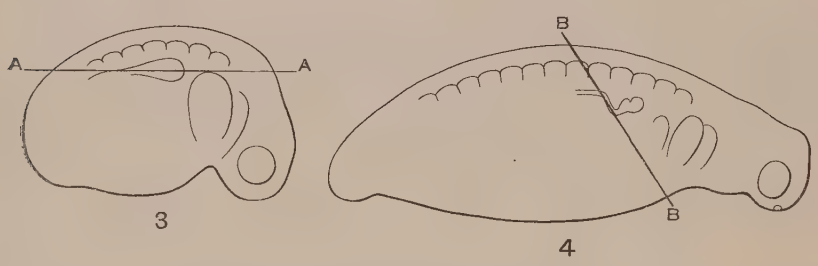
Figs. 1 and 2 Embryos in the stages used for operating. *PR*, pronephros located below the third and fourth myotomes. Figure 1, earliest stage (stage 30); figure 2, latest stage (stage 32).

Anaesthetics were unnecessary, and the slight motion due to the ciliated epithelium was controlled by holding the animal in the field with an operating needle. The body tissues in these early stages are easily distinguished from each other through slight differences in pigmentation, and, in addition, are so loosely bound together as to allow removal of the pronephric mesoderm without dislocating the cells of contiguous regions. In a few instances portions of the somatopleural layer ventral to the pronephric rudiment were included in the tissue removed, resulting in retarded development, in abnormalities, or even in total absence of the limb on this side (Harrison, '18).

[3] See Harrison, R. G., '18, Jour. Exp. Zoöl., vol. 25, no. 2, p. 417, footnote 9.

The general methods employed in operating are so well known that no detailed description of them is necessary here. The special technique required in removal of the pronephros and in the construction and measurement of the models will be described in later sections.

The pronephric swelling is one of the earliest and most clearly defined of the developing organs. Its position may be accurately located at a stage not long after the closing over of the neural folds, when the first eight pairs of muscle plates may be seen, and, like these, it differentiates in an anteroposterior direction. It is found in the region immediately underlying the

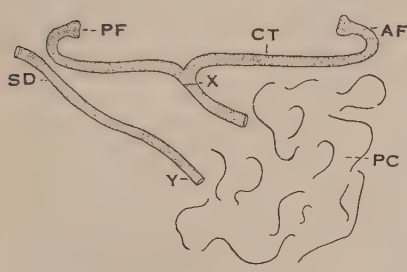


Figs. 3 and 4 Embryos showing the first looping of the tubule, due to rapid growth of the cells just posterior to the nephrostomes. Figure 3, *a–a*, original axis of the pronephric rudiment. Figure 4, *b–b*, direction of first bend of the tubule.

third and fourth myotomes as a bulbous thickening tapering posteriorly into a short thickened ridge. Operations at this period, although having the advantage of not interfering with developing nerves or blood-vessels, are inadvisable, since the mesoderm is still so compact that excision results almost invariably in the removal of more than the pronephric rudiment. In succeeding stages, the delimitation of the segmental duct progresses, and the original bulbous enlargement becomes pitted in in two places on its coelomic border, establishing the nephrostomal openings into the anterior and posterior funnels. These two openings lie opposite the midline of somites three and four. At the same time the tubule, which originally lies along the longitudinal axis of the body just below the muscle plates (fig.

3, *a*—*a*), lengthens rapidly in the region of the fourth myotome, and bending outward and downward in an acute angle over the upper surface of the yolk (fig. 4, *b*—*b*), forms a U-shaped loop. A little later this is bent over anteriorly, and may even come to lie slightly farther forward than the anterior nephrostome.

The pronephros of Amblystoma differs from that of certain of the Anura in the possession of two instead of three nephrostomal canals,[4] and in the absence of the common chamber or 'pronephric pouch,' the funnels, instead, narrowing directly



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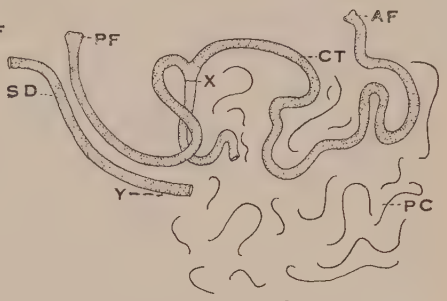

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Figs. 5 and 6 Diagrams showing the region where greatest growth occurs in the early and late stages of development of the pronephros. Figure 5, condition before the coiling of the longitudinal tubules connecting the funnels. Figure 6, growth of these tubules in the older kidney. *a.f.*, anterior funnel; *p.f.*, posterior funnel; *c.t*, longitudinal tubule; *s.d.* segmental duct; *x-y*, region of greatest growth during early development resulting in the formation of the ventrolateral portion of the pronephric coil, *p.c.* Shaded areas drawn from wax models, pronephric coil indicated by curved lines.

into the U-shaped tube just described. With further multiplication of the cells just below the funnels, two longitudinal tubules are established (figs. 5 and 6, *c.t.*, and fig. 17, *L.T.*), separating the anterior and posterior nephrostomes from each other and from their original point of junction with the looped tubule (*x*), as they grow. This growth is at first a very slow process as compared with that of the U-shaped portion. In the latter region the active proliferation of cells results in a rapid

[4] One instance is on record of the presence of a third funnel on both sides in an Amblystoma larva. See Field ('91).

coiling of the tubule, the early increase in size of the kidney being limited mainly to this region, between the connecting tubule and the proximal end of the segmental duct (figs. 5 and 6, *x* to *y*). This eventually forms the ventrolateral region of the fully formed organ, which will again be referred to in connection with the discussion of edema.

In still later stages[5] the nephrostomal canals and their connecting tubule also elongate, and are thrown into loops and folds (fig. 6, *l.t.*), retaining their dorsal position and extending slightly laterally over the coils already formed. This portion may then be termed the dorsolateral region, as contrasted with the ventrolateral portion already mentioned. That part of the tubule which is a direct continuation of the segmental duct never becomes strongly convoluted, but retains its original position along the ventrolateral boundary, slanting obliquely toward the dorsal surface over the kidney from the anterior margin. Minor folds may occasionally occur along its course. Increase in growth is outwardly evidenced by a more and more pronounced swelling in the pronephric region. Operated specimens may be easily distinguished from normal animals, even after healing is complete, through the absence of this thickening on the operated side. Posterior to the pronephric coils, the segmental duct extends backward along the body just below the ventral surfaces of the muscle plates. The junction between pronephric coils and the proximal end of the segmental duct is always in the immediate region of the posterior funnel. With the subsequent downgrowth of the myotomes the formation of the shoulder-girdle and anterior limb buds, the pronephros becomes partly covered, and comes to lie deeper in the body, and nearer the midline of the embryo. The edges of the myotome also extend downward over the segmental duct, making its removal in this stage extremely difficult.

[5] Excised kidneys in the older stages may be slightly stained, and the capsular nuclei, thus made visible, removed. The tubules may then be easily uncoiled for observation in water or weak alcohol. Oil is an unsatisfactory medium, both for examination and preservation, as it not only increases the brittleness of the tubules, but renders them too transparent for clear definition.

Parallel with this development, although not appearing at so early a stage, the rudiment of the glomerulus is formed. This first appears as a thickening in the opposite or splanchnic wall of the coelom, extending over an area as long as the distance between the anterior and posterior funnels. Vascular cavities soon appear, and at an early stage these become continuous with branches of the dorsal aorta. The arterial supply is derived directly from this source; the venous supply from the postcardinal vein, which enlarges in the region of the pronephros, forming a large venous sinus, into which the fully developed kidney projects. The tubules are thus continually bathed in the blood returning from the posterior part of the body (Field, '91).

BILATERAL EXCISION OF PRONEPHRIC RUDIMENTS

Mode of operation

The first experiments consisted in the removal of the pronephric rudiments on both sides, to test the functional necessity of these primary organs in the life of the embryo.

In the largest proportion of cases, a period of several hours, or even a day, was allowed to elapse between the removal of the right and left pronephros. However, the two excisions may follow each other immediately without incurring serious results.

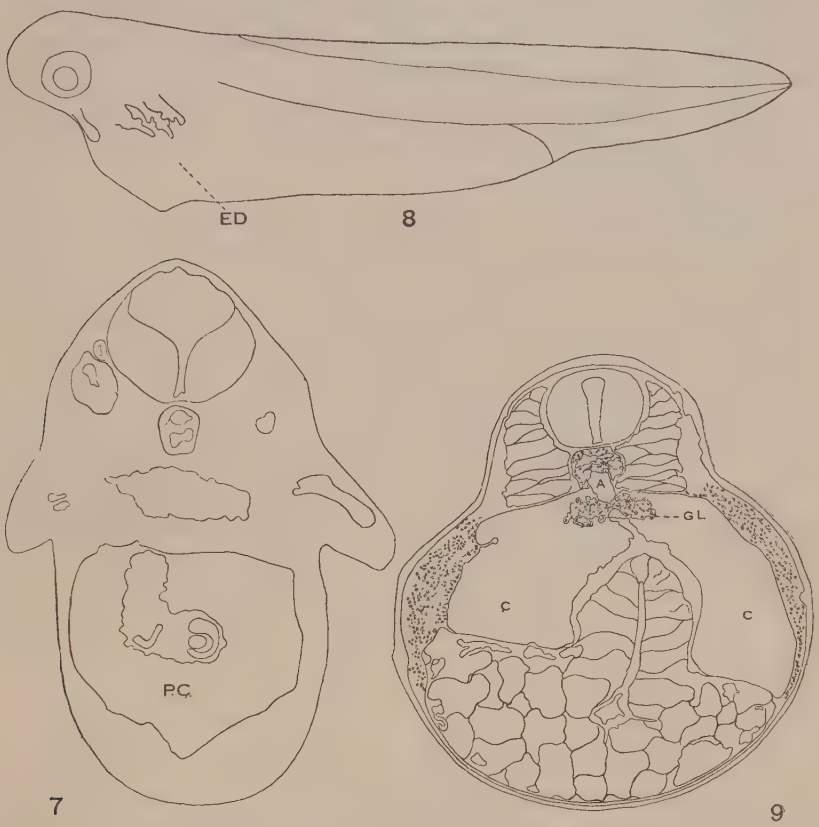
Sharp-pointed needles, inserted in glass rods, were substituted for the more generally used iridectomy scissors. Controls were kept under identical conditions of light, temperature, water, etc. Two methods were employed in removing the pronephros. In the first, three straight cuts were made, one beneath and one along each side of the pronephric swelling. The flap of ectoderm thus defined was loosened from the underlying mesoderm, and the organ removed from below. In the second and more satisfactory method, a single incision was made, dorsal to or immediately over the thickening, the tubule raised upward from below, pulled outward, and excised. In loosening the nephrostomal surfaces of the funnels, as much of the tissue in a dorsomedian direction was removed as seemed possible without disturbing the splanchnopleuric wall, since in this region the glomerulus normally arises.

Larvae in which the triple-incision method was used healed much more slowly than those to which the second method was applied, since contraction of the cut edges left a gaping wound much greater in extent and permitted more oozing of the yolk. On the other hand, when only one cut was made, a critical inspection of the excised tubules was necessary to make sure of total removal. In the majority of cases the incision is entirely healed at the end of an hour and a half.

Effect of bilateral removal

Most conspicuous of any of the postoperative conditions resulting from bilateral excisions was a pronounced edema, particularly in the anteroventral region. It is well known that edema of the amphibian embryo commonly occurs as the result of a variety of causes. Narcotized embryos reared in a solution of acetone-chloroform (chloretone), though structurally normal in other particulars, show a slight edema and pericardial effusion as a result of weakened heart action (Harrison, '04). More pronounced abnormalities result when early developmental stages are exposed to the heat of direct sunlight, or may be induced experimentally by exposing the embryos to radium rays (O. Hertwig, '11). McClure ('19), in his recent work on edema in anuran larvae, draws attention to the "less extensive tubular complex which normally occupies a dorsolateral position in the pronephros, and into which the nephrostomal canals directly open," and the "tubules which normally constitute the greater portion of the kidney and which occupy a medial and ventral position." From a study of the histological conditions existing in edematous frog larvae, he concludes that there is a functional as well as a morphological difference between these two regions, for in all of the embryos in which edema had become apparent, the ventrolateral tubules were either entirely absent or but poorly developed. From this he argues that deficiency in the ventrolateral tubules alone may be the cause of edematous conditions.

In bilaterally operated Amblystoma larvae, the swelling which first appeared in the region of the wounds progressed gradually forward, the pericardial cavity soon becoming enlarged (figs. 7



Figs. 7 and 8 Section and entire sketch of embryo, from which both kidneys had been removed, to show pericardial effusion. Figure 7, section through pericardial (*p.c.*) region. Figure 8, camera drawing of embryo, showing swelling in the heart region.

Fig. 9 Section through an embryo from which both kidneys had been removed. Both glomeruli (*gl*) are present, extending out into the enlarged coelomic cavities (*c*); *A*, aorta.

and 8). Later the fluid caused a pronounced distention of the abdominal cavities (fig. 9), and in extreme cases the gills also became swollen and distorted. Slowing or entire absence of circulation accompanied this condition and sloughing of the

ectoderm was not infrequent. Microscopic examination of sections through edematous embryos showed the tissues of the body to be in various stages of degeneration. Pressure of the accumulated fluid often forced the intestine ventrally or to one side and the fibers of the muscle plates were separated by large vacuoles. The muscle fibers themselves also became vacuolated, and in extreme cases the whole region was reduced to a spongy mass of irregular fibers with scattered nuclei.

Although the splanchnopleural mesoderm which gives rise to the glomeruli had been left intact during the operations, the question nevertheless arose as to whether normal conditions of development would obtain for these organs in the absence of other parts so closely allied with them. Sections made through the operated region in embryos killed four days and six days after double excision showed capillary tufts extending out into the much-dilated coelomic cavity (fig. 9, *gl*). The tubular region cannot, therefore, be considered to exert any influence in the nature of a formative stimulus on the development of the glomeruli, since these parts of the system arise quite independently. Furthermore, the presence of the glomeruli in these operated cases would tend to strengthen the view supported by McClure ('19) that the glomerular filtrate, given off directly into the coelomic cavity, collects here in excess, producing the typical edematous condition already described. These embryos also showed well-developed anterior and posterior funnels, extending laterally into the regions from which the tubules had been removed, and ending blindly there. Segmental ducts were present, in some cases the lumina being flattened dorsoventrally, in other instances the cells of these tubules showing marked signs of atrophy. The condition of the funnels and segmental ducts after extirpation of the kidney will be dealt with more fully in connection with the question of unilateral removal.

Efforts were made to bridge over the interval between the operations and the beginning of functional activity of the mesonephros. The first means applied was that of pricking the body wall as soon as abnormal distention was evidenced. The larvae

were immersed in 0.4 per cent NaCl in an attempt to balance the loss of essential salts through the escape of the glomerular filtrate. Although slightly stimulated heart action resulted, probably due both to the stimulus of operation and to relieved pressure in the pericardial cavity, only temporary benefit was derived in this way, for, with the accumulation of new fluid, the former pathologic condition was restored, and death followed after a short interval. It is quite possible that if a sufficient number of experiments were made, varying the constituents of the solution in which these larvae were kept, a satisfactory medium might be found for prolonging the life of these animals. A determination of the optimum salt percentages of such a solution has not yet been undertaken.

The second means employed was the transplantation of the pronephric rudiment to the region of the mesonephros. It was by this means possible to test the capacity for reestablishment of function, through union with the segmental duct. In a series of thirty embryos, the right head kidney, without the ectodermal covering, was placed under the skin farther back on the same side. Twenty-four hours later, the left pronephros was removed from those embryos which had responded well to the first operation and were apparently recovered. The transference and proper orientation of the kidney in these operations was easily accomplished and the wounds healed entirely in the usual short time, but the general edematous condition common to embryos on which only the bilateral excision had been performed subsequently developed. With the exception of a few which were preserved and sectioned at the end of a week, all of this series died within twelve days, showing no indication of resumption of function by the transplanted tubule. The transplanted tubule still retained its identity, although as a general rule the cells were pressed together in a solid mass, and only in a few instances a distinct lumen was visible. Removal of the pronephros resulted here in the partial or total atrophy of the segmental duct, to which attention will be called in greater detail below. However, in cases where the transplanted tubule had been placed in the immediate region of the segmental duct, no connection was

restored between these two components, nor did the duct, posterior to the transplant, give any evidence of functioning.

A slightly different method was applied in a third series of experiments. The pronephros was not alone transplanted, but was taken together with the overlying ectoderm, the surrounding mesoderm, and even small portions of the ventral myotomal walls. This transplant was procured from another animal, and was transferred into a previously prepared incision and held in place until healed. On the next two successive days the left and right pronephric rudiments were removed. No appreciable

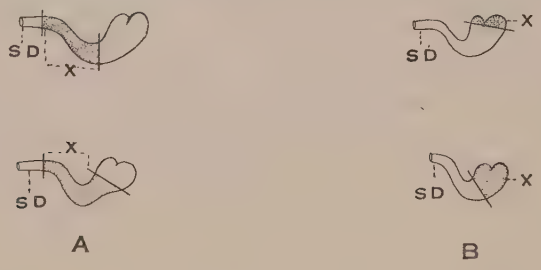


Fig. 10, A and B Diagrams to show the location and extent of operations made in removal of different segments of the embryonic pronephros. In A the segment removed, *x*, was a part or the whole of the U-shaped tubule anterior to the segmental duct. In B the segment removed, *x*, consisted of a part or the whole of the rudiment of the funnels.

difference was noted in the ensuing condition of this series, and it is safe to conclude that under these circumstances the excised tubule is unable to readjust itself and function in its new location.

Interruptions to the development of the pronephros by a less radical operation also go to strengthen the belief that the regenerative capacity of the kidney tubule is either very limited or very slow in taking place.[6] In a number of embryos from which one pronephros had been extirpated, a small portion of the opposite rudiment was also excised. The segments removed (*x*) were at two levels, as designated in figure 10, A and B. The funnels were undisturbed in one group (A), a short piece of the

[6] This does not apply to the coelomic epithelium which lines the nephrostomal opening, as will be shown in a later section.

first loop being removed, while in the second group (B), the funnel rudiment was cut off just anterior to the first bend. Of the twenty specimens used, all exhibited symptoms identical with those induced by bilateral excision.

The extent of regeneration which would occur in a defective tubule if the opposite kidney were allowed to remain intact is still a matter for investigation, but it is not improbable that a given portion of a tubule may possess a prospective potency which would insure the restoration of an excised section. However, in dealing with an organ where the demand for functional activity follows so closely upon this disturbance of normal condition, the requisite time for readjustment may be the factor lacking. Accumulation of excretory fluid may inhibit the regeneration which might be the normal consequence, if excretory activity were maintained by an undisturbed kidney. A fundamental difference thus places excision of the kidney in a category apart from the majority of regeneration or transplantation experiments upon the amphibian embryo which have been reported up to the present, for the effects consequent on extirpation and transfer of limb rudiments, optic vesicles, or nasal pits, though abnormal, are not of a nature to interfere with any of the vital functions of the embryo.

UNILATERAL EXCISION OF PRONEPHRIC RUDIMENTS

Conclusive evidence having been obtained as to the essential nature of the pronephros in the life of the embryo, a further study of the correlation of the development of this organ with that of the other components of the excretory system was then undertaken.[7] Unilateral excision of the pronephric rudiment served as a practical means to this end.

The technique of operating has already been discussed in the previous section, but a word of explanation is necessary regarding the controls used in this series. Since a more or less pronounced retardation in growth was the unavoidable consequence

[7] As has been previously stated, the glomerulus was found to develop normally even in the absence of the pronephric coil.

of such operations, the controls were always more advanced than the operated embryos, so that, for the comparison of the excretory organs, others had to be selected as described below (p. 373).

Postoperative effect on the embryo as a whole

Every outward evidence of successful readjustment to the new conditions imposed was shown by the operated larvae. Adverse symptoms, such as edema and general sluggishness, were absent, and, barring the slight retardation already mentioned, normal progress continued, except in those cases in which the limb bud was disturbed or entirely removed. The ectodermal surface which showed a slight concentration of pigment in the initial stages of wound healing gradually became indistinguishable from the surrounding regions, and differed from the opposite side only in the absence of the distention caused by the underlying pronephric coils.

Effect of unilateral excision on the remaining pronephros

The pronephros remaining after removal of one head kidney obviously takes over the function of excretion usually performed by the two organs. Beginning with the fourth day after excision, operated embryos were killed for observation each day for a period of two weeks. Sections showed distinct changes in the several remaining components of the excretory system, particularly in the head kidney functioning alone, the size of which was indicative of a marked compensatory hypertrophy. Since the controls taken from the same egg mass and carried along under the same conditions as those to which the operated forms were subjected invariably showed on sectioning a more advanced stage of development, the first step in determining the nature and extent of the change in the operated kidney was the establishment of a criterion for comparison of an operated with a normal embryo. An operated individual (PN 7) was chosen as a typical case and a large number of normal larvae of apparently the same age was examined to obtain

one in which the stage of development was identical. In many embryos where superficial features, such as length, breadth, and condition of limb and gill rudiments, were the same as those of PN 7, it was found on sectioning that the internal organs varied widely in degree of development. The normal larva finally selected (PN 7 d) tallied[8] not only in external measurements, but showed the several internal organs (retina, lens of eye, digestive tract, etc.) to be in a stage corresponding to those of PN 7.

As a further check against the possibility of error in the choice of a normal duplicate, a second duplicate was chosen, and the respective volumes of the kidneys of the two roughly compared by the following method:[9] On drawing-paper of uniform thickness the serial sections of the entire kidney of PN 7 d and of the second duplicate (PN 7 d, no. 2) were projected and the lumen of the tubule outlined. The drawings of each kidney were then carefully cut out. No attempt was made to assemble them in the form of a model, but the weight of the paper used for each was taken as a standard for comparison. The weight of PN 7 d was 2.35 grams and that of PN 7 d, no. 2, 2.26 grams, giving a difference of only 0.09 gram, or about 4 per cent—a variation so small as to be considered negligible. The larger normal kidney (PN 7 d) was used for comparison with the hypertrophied one in order to lessen the possibility of exaggerating the difference between the two.

After the normal duplicate (PN 7 d) had been selected, several methods were open for the determination of the nature and degree of the hypertrophy of the remaining pronephros in the embryo from which the organ on one side had been removed.

[8] The slight variation would tend rather to minimize the contrast than to accentuate it, since, if either, PN 7 d is the more advanced.

[9] In connection with the review of Kittleson's paper (see previous reference), I find that somewhat the same method was employed by him in his estimation of the relative surface areas and weights of the kidneys of rats. The weight in grams was reduced to square centimeters by estimating the average area in square centimeters of one gram of paper, and from this the total volume of the kidney was estimated.

Wax models of the unoperated right pronephros of PN 7 and the corresponding organ in PN 7 d were constructed by means of the Born method, at a magnification of 200 (figs. 18 to 21). Unlike the normal model, to which reference has already been made in an earlier section, these two are reconstructions of the lumen of the tubules without enclosing walls. The model of the kidney of the operated embryo not only showed a considerable increase in the thickness of the tubule as contrasted with that of the normal, but its length is also appreciably greater. This was determined by taking the average of five measurements. A flexible but inelastic cord was pinned along the surface of the wax for its entire course, and its length thus recorded. On each measurement the cord was pinned along a different surface, so that the data would be of a representative nature. For the model of the normal kidney the average length was found to be 155 cm., at a magnification of 200, with a probable error of ± 0.181: for PN 7, 188 cm., at a magnification of 200, with a probable error of ± 0.155, showing an increase of 21 per cent over the normal condition.

Microscopic examination of the tubules showed the walls in the normal organ (PN 7 d) to be relatively thick and made up of cuboidal cells. The hypertrophied tubules (PN 7) were thinner walled proportionately, the cells often flattened and elongated, and the lumen strikingly larger than that of the unoperated specimen[10] (figs. 22 and 23). Outline drawings were made of the outer and inner boundary of the walls of the hypertrophied and of the normal tubules at a magnification of 600 (figs. 11 and 12). A non-elastic cord was then pinned at frequent intervals along the inner lines, removed and measured, and the circumference obtained. Five different sections were used at different levels in each case, and in each section from three to five tubules were measured, making a total of twenty-one measurements for each kidney. The average measurement obtained for PN 7 d

[10] In a series of experiments reported by Detwiler ('18), the pronephros was often carried along in the transplantation of the limb rudiments. The enlargement of the undisturbed pronephros and its contrast with the normal condition may also be seen on examination of his plates (Jour. Exp. Zoöl., vol. 25, 1918, pl. 3, figs. 18 and 19).

was 78 mm. (0.13 mm. in actual size), as contrasted with 130 mm. (0.216 mm. in actual size) for PN 7—a fact suggesting the large percentage of increase in functional capacity of the two kidneys determined and described below. From these projections also the thickness of the walls was estimated by taking the average of sixty measurements in each kidney. The average thickness of the wall of the normal kidney is 10.9 mm. at a mag-

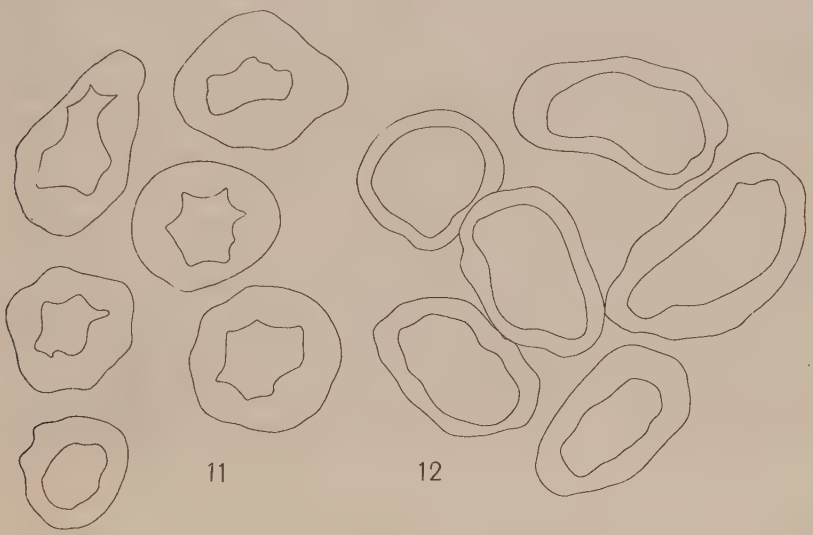


Fig. 11, PN 7 d Normal tubules with thick walls, and cells bulging into the lumen. × 240.

Fig. 12, PN 7 Hypertrophied tubules with large lumens and thinner walls. × 240.

nification of 600, or 0.0181 mm. in actual measurement. For the hypertrophied kidney, the average thickness is 7.8 mm. at a magnification of 600, or 0.013 mm. in actual measurement.

Having the length of the two kidneys, determined from the models, and considering each kidney as a simple cylinder, with the average measurement just obtained as its circumference and the length as its height, the areas of the inner surfaces in each were computed. In the normal kidney the area of the secreting surface was found to be 1.007 sq. mm. as contrasted

with 2.037 sq. mm. in the compensating organ—an increase of more than 100 per cent (table 1).

A comparison of the volumes of the cells making up the walls of the two kidneys is likewise of great importance in determining the nature of the response brought about by a unilateral operation. Although in the calculation of the surface area of the tubules it seemed sufficiently accurate to regard them as cylinders with the average circumference as their boundary, it did not seem possible to apply such a geometrical method in estimating the volume of the walls, for the cells (figs. 11 and 12), especially in the normal kidney, often bulge out into the lumen, making this, as seen in projected outline, very irregular. The method already described in the selection of a normal model was again

TABLE 1

Showing the area of the inner or secreting surfaces of the normal kidney, PN 7 d, and the hypertrophied kidney, PN 7

SERIES NUMBER	INNER CIRCUMFERENCE (× 600)	OUTER CIRCUMFERENCE (× 600)	LENGTH (× 600)	AREA OF INNER SURFACE (× 360,000)	ACTUAL AREA OF SURFACE
	cm.	*cm.*	*cm.*	*sq. cm.*	*sq. mm.*
PN 7 d (normal).....	7.8	13.6	465	3627	1.007
PN 7 (hypertrophied)....	13	18.6	564	7332	2.037

used (p. 373). Paper of uniform weight was obtained, and to insure further accuracy, section number one of the normal kidney was projected on one half of a sheet, section one of the hypertrophied organ on the same sheet of paper, and so on through the series. The walls of the two kidneys were then cut out, and the aggregate of each weighed separately. The paper representing the walls of PN 7 d weighed 20.39 grams that of PN 7, 33.4 grams, showing an increase of 63 per cent in the weight of the latter. Since the weight in this case is in direct proportion to the volume, the hypertrophied kidney may then be considered as showing an increase of almost two-thirds beyond the normal. A count of the nuclei in each kidney, all the sections of which were cut at 10μ, shows a small percentage (16 per cent) of increase in the number of cells found in the larger kidney,

there being 1950 nuclei in PN 7 d and 2252 in PN 7. Although this may indicate a certain amount of hyperplasia, that is, increase in actual number of cells present, it is not large enough to account for the great percentage of increase in both secreting surface and in volume. This increase must be mainly attributed to actual hypertrophy or enlargement of the cells already present.

Effect of unilateral excision on the glomerulus

A study of the condition of the glomeruli subsequent to double excision was necessarily limited by the early death of the embryos. The occurrence of the glomerulus in these cases has already been noted. On examining larvae from which only one pronephros had been removed, both glomeruli were found to be invariably present. The one on the operated side, however, exhibited less uniformity in size and shape than the normally functioning one. In some cases the outer layer of the glomerulus and the epithelial lining of the body wall had coalesced, this being the case not only on the operated, but on the normal side as well. On the operated size, however, the distance to be bridged is very greatly increased, since the absence of the pronephric swelling increases the width of the coelomic cavity there (fig. 13).

Since the development of this part of the kidney unit is quite independent of the presence of the tubular portion, its functional activity can be counted on to continue undisturbed. No hypertrophy in glomerular structure, then, would be anticipated, nor was such found to be the case, for the structural conditions existing allow free passage of the filtrate ventrally from one side to the other through the coelomic cavity. Filtration continues on both sides, the demand for increased physiological activity falling only on the tubules of the unoperated side.

Effect of unilateral excision on other components of this system

Removal of one head kidney has a widely varied effect on the formation of the segmental duct on the operated side. The process of development of the non-functioning ducts took place

irregularly, and, in general, only to a limited extent. As shown in table 2, every gradation occurred, ranging from a condition in which the lumen, though small and flattened dorsoventrally, appeared throughout the entire length (A 51, 5 days) to a condition where only the occasional presence of a few degenerating cells indicated the location of the atrophied rudiment (A1, 10 days).

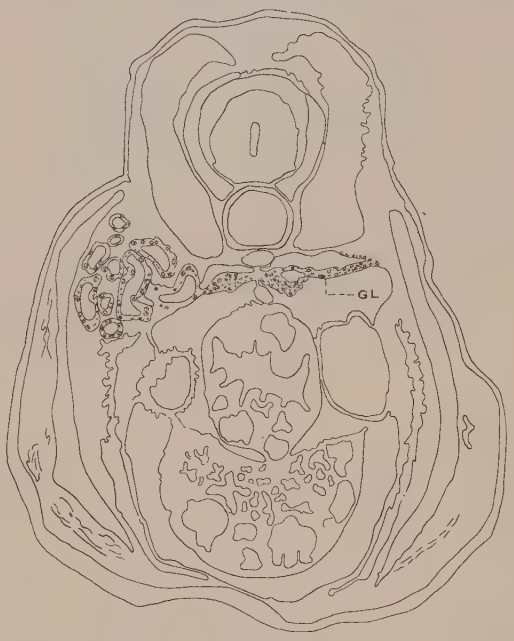
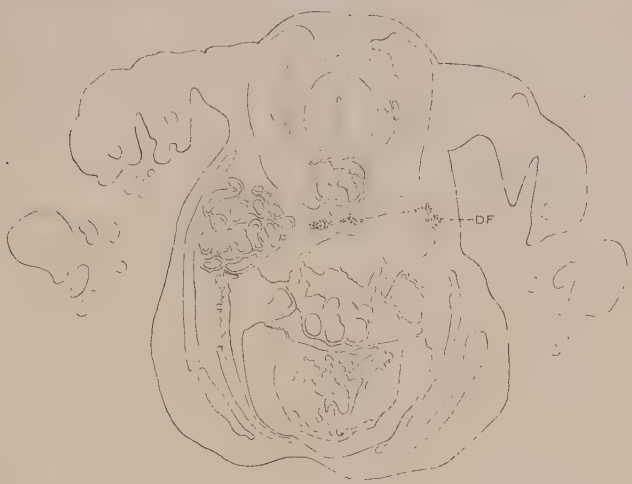


Fig. 13, PN 2 Showing the increased width of the glomerulus (*gl*) on the operated side. Description in text. × 40.

Increased activity of a single kidney also has a definite effect on the segmental duct of that side. Cross-sections of the duct of an individual with unilateral operation, when compared with either of the ducts of a normal larva of the same age, show a marked increase in diameter.

Although in the excision of the pronephric rudiment great care was taken to remove as much of the somatopleural mesoderm as seemed feasible without disturbing the underlying tissues, an examination of the operated embryos showed that a large num-

ber possessed well-developed anterior and posterior nephrostomes ending blindly (figs. 14 and 15 and table 2). This would indicate that the adjacent coelomic endothelium possessed the



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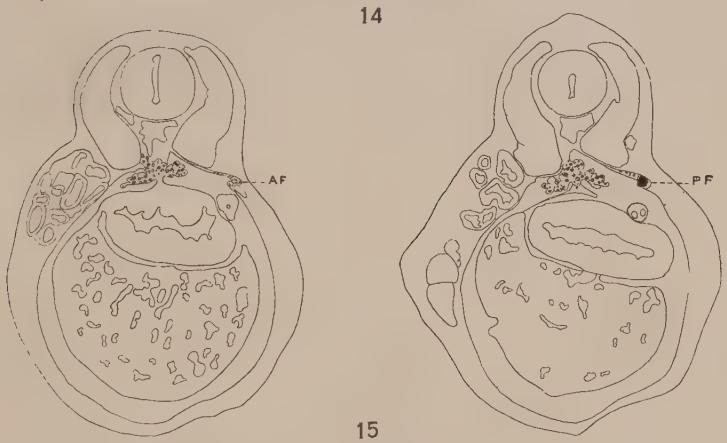

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Fig. 14, PN 2 Showing double anterior funnel (*d.f.*). × 30.
Fig. 15, PN 7 A, regenerated anterior funnel (*a.f*), on the operated side. B, regenerated posterior funnel (*p.f*) on the operated side. × 30.

capacity for regeneration of this portion of the organ, even though, as we have already seen, this property is not shown by the tubules themselves. Of the fifteen embryos tabulated

TABLE 2

Showing condition of ducts, nephrostomes, and glomeruli in embryos from which one pronephros has been removed

SERIES NUMBER	AGE	DUCT ON OPERATED SIDE	DUCT ON UNOPERATED SIDE	NEPHROSTOMES	GLOMERULI
	days				
A 54	4	Canalized at intervals, flattened	Round, canalized, definite	Anterior present, posterior absent	Both present
A 51	5	Small, canalized, flattened	Large, round, canalized	Anterior present, posterior present	Both present
A 51	6	Small, flattened, atrophied posteriorly	Large, round	No anterior, posterior present	Both present
A 54 no. 1	7	Small, canalized	Large, round	Anterior present, posterior absent	Both present
A 54 no. 2	7	Present anteriorly atrophied posteriorly	Large, round	Anterior present, posterior present	Both present
A 54 no. 3	7	Present anteriorly, atrophied posteriorly	Large, round	Anterior absent, posterior present	Both present
A 8	9	Small, slightly canalized	Large, round	Small anterior, no posterior	Both present
A 1	10	A few degenerating cells	Large, round	Anterior present, posterior absent	Both present
A 1.	11	Small, irregularly canalized	Very large	Anterior present, posterior present	Both present
PN 2	17	Very small but canalized posteriorly	Large, round	2 anterior, 1 posterior	Both present
PN 3	29	Small, discontinuous anteriorly	Large, but flattened anteriorly	Anterior present, posterior present	Both present
PN 4	15	Very small, (whole mount)	Large, (whole mount)	No anterior, posterior present	Both present
PN 5	21	Small (whole mount)	Large, (whole mount)	No anterior, posterior present	Both present
PN 6	21	Small anteriorly, central portion discontinuous	Flattened anteriorly	Anterior present, posterior present	Both present
PN 7[1]	9	Small anteriorly, none posteriorly	Large, round	Anterior present, posterior present	Both present

[1] For PN 7, 9 days, see figs. 18 and 20.

(table 2), twelve had well-formed anterior, and twelve had posterior nephrostomes. In one instance (PN 2, 17 days) the anteror nephrostome had doubled, suggesting the three pronephric openings normally found in anuran larvae (fig. 14 and table 2).

A study of the development of the mesonephros in operated animals will be the subject of further work.

EFFECT OF REMOVAL OF THE HEART ON THE DEVELOPMENT OF THE GLOMERULUS

In a series of experiments dealing with the effect of removal of the heart on certain other organs of the embryo, Doctor Harrison removed the rudiment of the heart in larvae of stages 29 to 30. Through his kindness, these embryos were made available to me for a study of the effect produced on the glomeruli. With the incoming of new material (March, 1920), these cases were further augmented by additional experiments.

The glomerulus in Amblystoma punctatum, as has been stated in an earlier section, normally begins to differentiate from cells of the splanchnopleural wall below and at each side of the aorta, in stage 36. Within these clusters of cells vacuolated areas soon appear, and in a short time connect with the aorta.[11]

In embryos from which the heart has been removed before any contraction of the cardiac muscles occurred, the initial development of the cell groups is normal. However, as the connection with the aorta is established, the more or less compact nature of the tufts can no longer be maintained, but from pressure of the blood plasma which has collected in and is distending the blood-vessels, the vacuolated centers of the glomeruli are torn apart. As this accumulation of fluid increases, the outer walls of the tufts become more and more flattened, and consequently less easily distinguishable from the wall of the aorta, with which they are still continuous, finally losing their identity as separate organs. It is of interest, however, to note their early formation under these circumstances as additional proof of their independent power of development.

[11] A detailed description of this process together with plates is given by Field, '91 (pl. 1, figs. 8, 9, 10; pl. 6, figs. 48, 49, 50, 52, etc.).

SUMMARY AND CONCLUSIONS

From a study of the results obtained after bilateral and unilateral extirpation of the head kidney and of the heart rudiment of Amblystoma larvae, the following conclusions may be drawn:

1. Conditions ensuing on bilateral removal of the pronephros show clearly that this organ is necessary to the life of the embryo, although the presence of one pronephros suffices to keep the organism alive and in a healthy condition. All embryos from which both head kidneys had been extirpated died within from eight to twelve days, evidencing during that interval weakened heart action, edema, and effusion into the pericardial and abdominal cavities. Pricking the body wall to relieve the edematous condition proved ineffective.

2. Double extirpation does not affect the normal development of the glomeruli. These appear in embryos killed four days after the operation.

3. The pronephros remaining after the removal of one head kidney takes over the function of excretion usually performed by the two organs, and, concomitant with the increased physiological activity, presents marked morphological changes.

4. The adjustment consequent on unilateral removal consists not in the regeneration of the lost part, but in compensatory hypertrophy of the remaining organ, a response which has long been known to occur in the adult kidney and in other glandular organs, both paired and unpaired.

5. The area of the secreting surface in the hypertrophied kidney shows an increase of over 100 per cent when contrasted with the normal (2.037 sq. mm.; 1.007 sq. mm.).

6. The cubic content of the mass of cells constituting the hypertrophied kidney as shown by their relative weight is increased 63 per cent above the normal.

7. The length of the tubules shows an increase of 21 per cent.

8. The number of nuclei in the hypertrophied kidney exceeds that of the normal by 16 per cent, due to the occurrence of a small amount of hyperplasia.

9. In single, as well as in double, extirpations, the glomerulus develops normally in the absence of the pronephric tubules.

10. Anterior and posterior nephrostomal funnels are regenerated from the coelomic endothelium in a large proportion of operated embryos.

11. The segmental duct on the operated side shows great variation in development, ranging from a condition in which the lumen, though small and flattened dorsoventrally, appears throughout the entire length, to a condition where only the occasional presence of a few degenerating cells indicates the location of the atrophied duct.

12. Increased activity of a single kidney also has a definite effect on the segmental duct of the same side. Cross-sections of the duct of an individual with unilateral operation, when compared with either of the ducts of a normal larva of the same age, show a marked increase in diameter.

13. In embryos from which the heart rudiment has been removed in a very early stage, the initial development of the glomeruli is normal. Subsequent distention of the aorta tears the cells apart and they soon lose their identity as lateral capillary tufts.

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PLATES

ABBREVIATIONS

A, anterior
AF, anterior funnel
LT, longitudinal tubule
P, posterior
PC, pronephric coil
PF, posterior funnel
SD, segmental duct

PLATE 1

EXPLANATION OF FIGURES

16 Model of young normal pronephros, ventrolateral view. × 125.
17 Model of young normal pronephros, dorsolateral view. × 125.

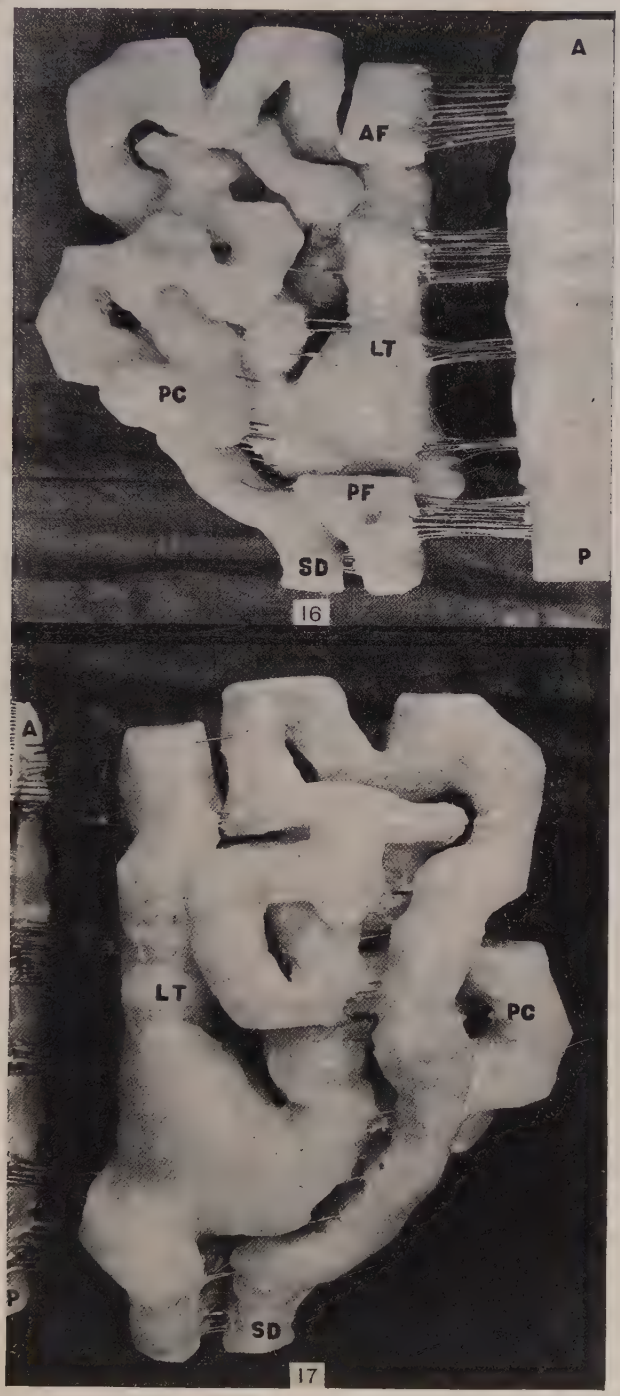
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PLATE 2

EXPLANATION OF FIGURES

18 Model of hypertrophied pronephros, ventrolateral view (PN 7).

19 Model of normal pronephros, of same age as figure 18, ventrolateral view (PN 7, d).

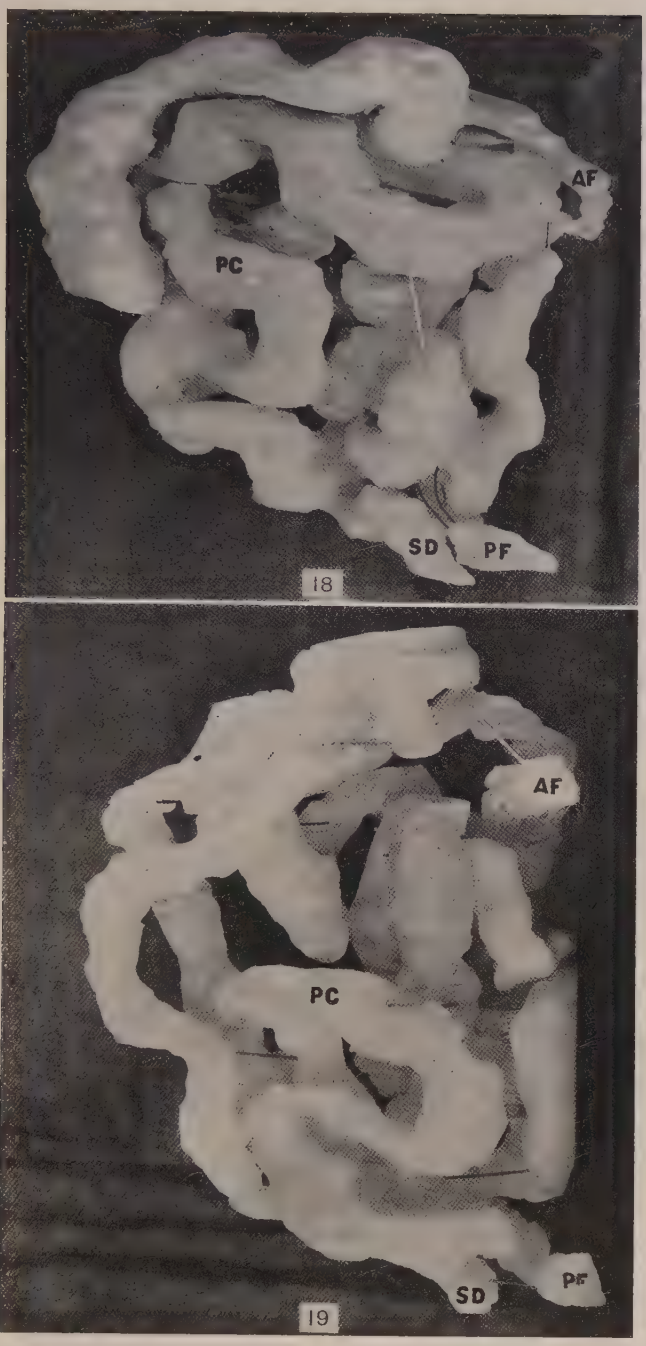
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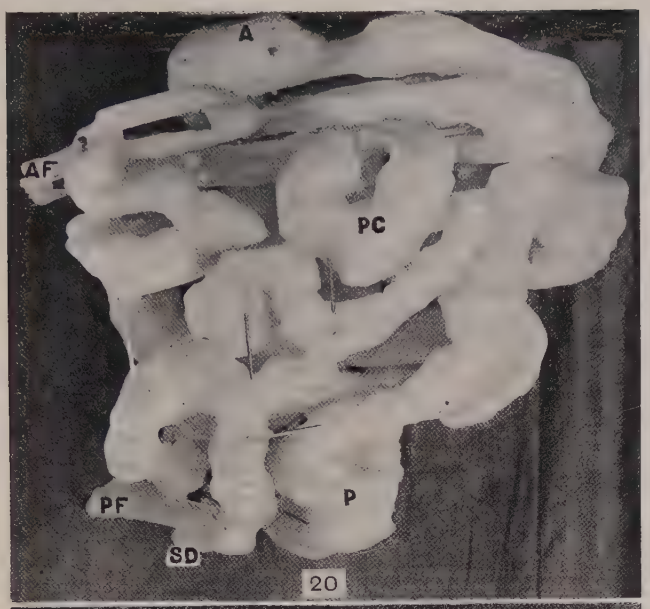
PLATE 3

EXPLANATION OF FIGURES

20 Model of hypertrophied pronephros, dorsolateral view (PN 7).

21 Model of normal pronephros, of same age as figure 20, dorsolateral view (PN 7, d).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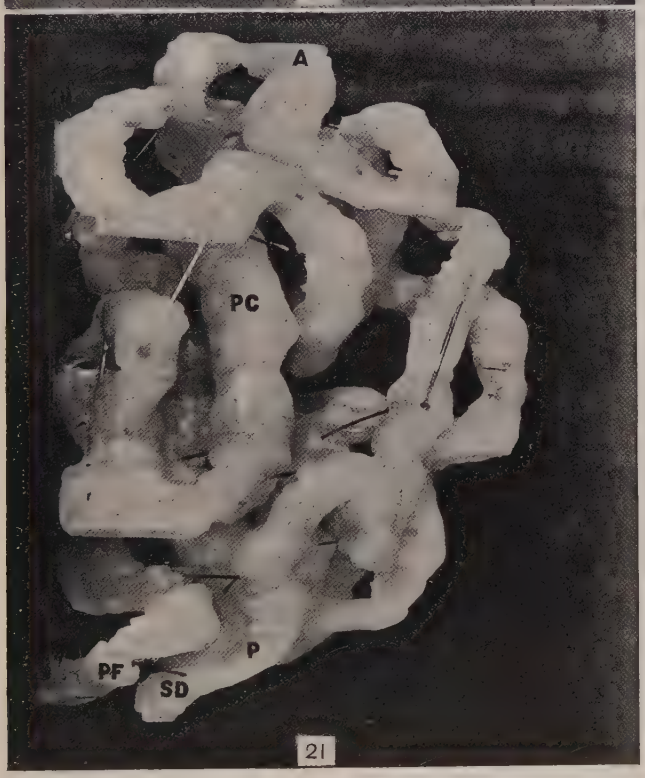
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PLATE 4

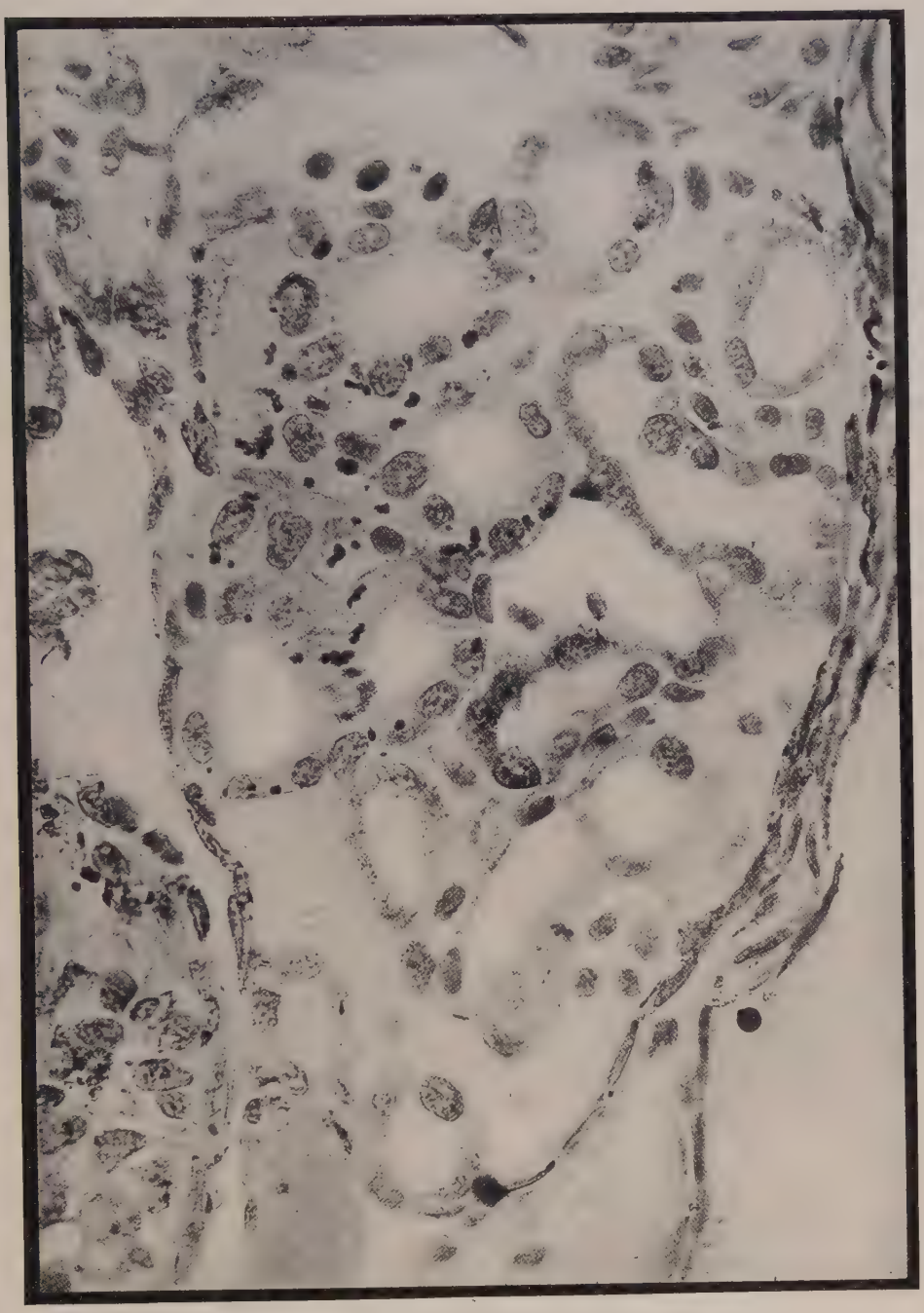
EXPLANATION OF FIGURE

22 Section of hypertrophied pronephros (PN 7). × 400.

PLATE 5

EXPLANATION OF FIGURE

23 Section of normal pronephros (PN 7, d). × 400.



Reprinted from the Proceedings of the Society for Experimental Biology and Medicine
1921, xviii, pp. 240–241.

119 (1701)

The early effects of conjugation on the division rate of Spathidium spathula.

By LORANDE LOSS WOODRUFF and HOPE SPENCER.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Conjugation occurred readily in a pedigree culture of *Spathidium spathula* and therefore experiments were started to determine the effects of fertilization in the life history of the organism. During the first six months of the work, more than sixty lines were derived directly or indirectly from the parent line by conjugation. Some of the exconjugant lines studied represent the F_1, F_2, F_3, and F_4 generations. All the lines which are compared were bred under identical cultural conditions.

A comparison of the number of generations attained by each exconjugant line with that attained by its parent line during the first 15 days after the former's origin gives the following results. Forty-two exconjugant lines produced more generations, eight produced less generations and two produced essentially the same number of generations as their respective parent lines. The various cases in which the parent line did not survive the first fifteen days after the exconjugant line was derived from it are not comprised in these data. If such cases were included it obviously would increase the number of plus cases of exconjugant lines.

Analysis of the data thus far obtained inevitably leads to the conclusion that the exconjugant lines of this pedigree culture of *Spathidium*, under the conditions of the experiment, exhibit, in the great majority of cases, a higher division rate for the first fifteen days after conjugation than the parent lines.

The evidence to date also indicates that exconjugant lines which are derived from old parent lines (*i.e.* from lines which have undergone many generations since conjugation) show a relatively greater increase in the division rate, during the first fifteen days, as compared with the parent lines, than do exconjugant lines which are derived from young parent lines (*i.e.*, from lines which have more recently conjugated).

The complete paper will appear in the *Journal of Experimental Zoölogy*.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1921, xviii, pp. 303–304.

156 (1738)

The survival value of conjugation in the life history of Spathidium spathula.

By LORANDE LOSS WOODRUFF and HOPE SPENCER.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

In a former communication it has been shown that in our pedigree cultures of *Spathidium spathula*, exconjugant lines, in the great majority of cases, exhibit a higher rate of division during the first fifteen days than the parent lines.[1] The purpose of the present paper is to summarize briefly the results to date with respect to the effects of conjugation as exhibited later in the life history of the pedigree lines.

1. Conjugation in the majority of cases increases the length of life of the exconjugant line, so that it lives after the death of the parent so-called "non-conjugant" line. This is shown by the fact that of forty-seven exconjugant lines, thirty lines lived longer; four lines lived to essentially the same date; and thirteen lines died before their respective parent lines.

2. The total number of generations attained by the exconjugant exceeds those attained by the parent from the date when the exconjugant arose to the death of the parent in about eighty per cent. of the lines. Of fifty-two comparable lines, forty-one lines exceeded their respective parent lines in number of fissions; two practically equalled; and nine lines did not attain so many generations. The fact is evident that conjugation increases the number of fissions to a total which could not otherwise have been reached.

3. If the period be considered during which both lines were alive, the results are even more conclusive. Forty-four lines at-

[1] L. L. Woodruff and Hope Spencer, Proc. Soc. Exp. Biol. and Med., 1921, xviii, 240–241.

tained more generations than the parent; four equalled the parent; and four completed a smaller number of fissions. Thus over eighty per cent. of the exconjugant lines attained more generations than their respective parent lines.

4. By analysis of the data from an entirely different angle the same general conclusion is apparent. The total number of generations attained before the F_1 generation appeared, plus those from the F_1 to the origin of the F_2, and so on to the F_5 generation, amounts to date to from 450 to 550 generations, according to the series followed. The first line reached 234 generations without conjugation, therefore by conjugation it thus far has been possible more than to double the number of fissions obtainable without conjugation.

In brief, all the data thus far secured indicate that in this pedigree culture, under the conditions of the experiment, the survival value of conjugation in the majority of cases is marked—the exconjugant lines exhibiting a higher division rate and outliving the non-conjugant lines.

The complete paper will appear in the Journal of Experimental Zoology.

THE SPERMATOGENESIS OF LEPISMA DOMESTICA

BY

HARRY H. CHARLTON

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A DISSERTATION

presented to the Faculty of the Graduate School of Yale University,
in candidacy for the Degree of Doctor of Philosophy

1920

Reprinted from JOURNAL OF MORPHOLOGY, Vol. 35, No. 2, June, 1921

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, MARCH 28

Reprinted from JOURNAL OF MORPHOLOGY
Vol. 35, No. 2, June, 1921

THE SPERMATOGENESIS OF LEPISMA DOMESTICA

HARRY H. CHARLTON

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SIX PLATES (NINETY-FIVE FIGURES)

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INTRODUCTION

Cytologists have long found the class Insecta to be a very fertile field for investigation, but the first order and, according to many, the most primitive, namely, the Thysanura, seems to have been neglected. According to Harvey ('16), only three papers dealing in any way with the cytology of any closely

related forms have been published. The writers, Claypole ('98), on Anurida maritima, Lecaillon ('01), on Orchesella villosa, and Willem ('00), on Podura aquatica, simply report isolated observations which are necessarily incomplete and limited to the class Collembola.

The Lepismatoidea have therefore never been made the subject of a cytological study, and it was in the hope that a survey of this primitive form would throw some light on the present-day cytological problems that this investigation was undertaken.

The completion of the study shows that, instead of the expected simplicity, the process actually is a complicated one, differing only here and there from that already described in other forms. These differences, however, are interesting and, together with the fact that it is the first cytological work in a new class of insects, warrant its presentation.

The work was done at the Osborn Zoological Laboratory at the suggestion of Professor Petrunkevitch beginning in the fall of 1916. During 1917–19 it was practically suspended except for an occasional day or so at Columbia University. It gives me pleasure to express my thanks to Prof. E. B. Wilson for his kindness in giving me laboratory privileges at Columbia and to Prof. Frank R. Lillie for facilities accorded at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1919.

Most of all, I am indebted to Prof. Alexander Petrunkevitch, first, for suggesting the problem and later for his unfailing help and criticism.

MATERIAL AND METHODS

Lepisma domestica, commonly called the fire-brat, from its frequently observed habit of running apparently unharmed over hot stones in bakeries, belongs to the class Thysanura of the order Lepismatoidea. It is a fairly common insect in New Haven, and can be kept alive in the laboratory for a considerable period. My method has been to keep them in large glass Stender dishes without covers, since the insects cannot climb up a clean glass wall, and to provide them with a cereal such as corn flakes

to eat. For moisture I have kept shallow dishes filled with moist filter-paper in the Stenders. In spite of these precautions, in the course of a month or so the creatures begin to show a shrinkage of the abdominal region and soon die with the posterior region shrunken fully one-half.

The insects were either killed in xylol or decapitated and the testes dissected out immediately in physiological salt solution. As soon as the body cavity was opened, some fixing fluid was introduced by means of a fine pipette. This renders the tissues more opaque and makes it easier to locate the gonads which are loosely surrounded by fat, as well as to cause better fixation.

For counting chromosomes, Bouin's fluid at 38°C. proved the best, but for general fixation of the cytoplasm as well as of chromatic structures nothing equaled Flemming's strong solution. In addition, Hermann's fluid, Benda's Flemming, 10 per cent formalin, Allen's modification of Bouin, Petrunkevitch's fluid, and Kopsch were tried out and had their special uses.

The testes dissected out, fixed, washed, and dehydrated, were imbedded in paraffin and cut into sections from 3 to 12 μ thick. Sections of 7 μ thickness were found to be very satisfactory for study, and in general this was the thickness used.

The stain used generally throughout was Heidenhain's iron haematoxylin without any counterstain. In addition, various counterstains were tried, and also, in an effort to get a selective stain for mitochondria, Benda's alizarin-crystal violet method. A modification of Cajal's silver-impregnation method by Hortega ('16), especially recommended for centrosomes, was given an inadequate trial with but fair results.

I have also examined a number of splended slides stained by the safranin-gentian-violet-orange G method, for which I am indebted to Dr. P. W. Whiting.

OBSERVATIONS

External sexual characters

During the spring months of March, April, and May, the adult insects are in the best condition for study, but since it takes some time, probably a year or more, to attain sexual maturity, the early stages may be studied at any time of year in young individuals.

It is comparatively easy to recognize the sexes by their external appearance. The female (fig. 2) has a long median ovipositor extending posteriorly which is quite prominent in the living insect. There is nothing comparable to it in the male (fig. 1), for the penis which could not possibly be confused with the ovipositor is more often retracted and not in view.

Male reproductive system

The testes (fig. 3), of which there are three pairs on each side of the middorsal line, occupy, in mature individuals, a considerable portion of the anterior two-thirds of the abdomen. The testes lie parallel to each other, extending in a ventroposterior direction, and each is connected by a short duct with the vas deferens, which passes as a straight duct posteriorly where it enlarges to form the seminal vesicle. From the seminal vesicle a similar duct extends, which soon enlarges considerably and, after bending upon itself a couple of times, opens into the base of the penis.

Spermatogonia

I have made a long and careful search for primary spermatogonia in the youngest material at my disposal, which consisted of insects only 2 or 3 mm. long, but have not been able definitely to identify them. It is therefore very probable that the primary spermatogonia occur only very early in the life-history. Munson ('06) has described an apical cell which produces early spermatogonia, but this, too, if present at all, would be found in exceedingly young individuals.

One does find cells in quite large numbers at the blind end of the testis, which differ from the ordinary spermatogonia in having large homogeneous nuclei with the chromatin condensed into a single dense mass and irregular in shape (fig. 28). I believe these to be immature Sertoli or nurse cells, for later on one finds such cells, only now they are larger, more elongated, and contain two to four chromatic bodies. Those at the region of the mature spermatozoa are much paler in color and may be wrinkled and twisted upon themselves, indicating perhaps degeneration.

The spermatogonia occupy a considerable part of the blind end of a tubule of the mature insect during the winter and early spring, and can be easily recognized by their position and by the arrangement of the chromatin in the form of clumps attached to each other by linin threads and grouped around the periphery of the nucleus. This arrangement (fig. 4 is a surface view) is the most common and probably represents a resting condition. Although I have not been able to count these clumps of chromatin, the number is easily seen to be more than the haploid, and each one probably represents a spermatogonial chromosome.

The nucleus of the early spermatogonium is quite large and almost equal in size to the nucleus of the growth period. The two or three spermatogonial divisions reduce the nuclear and cell size by apparently not allowing time for growth between divisions (figs. 8 to 11). In prophase the chromosomes are long and bent upon themselves and irregularly scattered throughout the nucleus; later they are drawn into the metaphase plate as shown in figure 5.

It is only in the larger and therefore the earlier spermatogonia that good counts of the chromosomes can be made. Figure 6 shows thirty-four chromosomes in a very clear metaphase plate. The chromosomes are of the curved-rod type, differing considerably in size, but close observation fails to show any chromosome or group of chromosomes behaving in any way differently from its neighbors.

In the telophase of the spermatogonial division the chromatin becomes granular and forms a more or less eccentric ring around

the nuclear wall (figs. 11 and 16). At a little later stage one commonly finds an irregular clump of chromatic material representing apparently two spermatogonial chromosomes lying against the nuclear membrane and retaining the haematoxylin stain (figs. 13, 14, and 15). For these chromosomes I shall use the term idiochromosomes, the name given by Wilson ('05) and meaning 'peculiar or distinctive chromosomes.'

In some presumably young cysts the spermatogonial cells are arranged in the form of a rosette, their median ends tapering toward a faintly marked open center and showing an archoplasmic mass or sphere (figs. 4 and 18). Hegner ('14), Meves ('97), and Shaffer ('17), as well as others, have described similar structures and considered them spindle remains. In some cases they figure them as extending from cell to cell. While this is true immediately after division when the spindle remains are very definite (fig. 7), it is not possible later to see any continuation or connection with similar bodies in adjacent cells.

In the spermatogonial region isolated cells are occasionally seen in division, but, strangely enough, the chromosomes are paired and look somewhat like tetrads (fig. 32). The cells themselves are much larger than the spermatogonia and contain but little cytoplasmic staining material in the form of a flaky mass at either end of the cell in which a dark-staining granule may be seen. If these represent division in the Sertoli cells, they are a very rare occurrence. In the older Sertoli cells I have occasionally seen evidence indicating division by amitosis.

The growth period

The stages of the growth period correspond fairly closely with the stages described by Wilson ('12). After the telophase, chromosomes of the last spermatogonial division break up and form a granular ring just inside the nuclear wall, the chromatin arranges itself as previously described in the form of clumps located on the nuclear membrane (Wilson's stage b, similar to fig. 13). In heavily destained material two of these are closely related, one of them being flattened against the nuclear mem-

brane and retaining the dark stain of the haematoxylin (fig. 15). In addition to the idiochromosomes, a similarly staining, small, spherical granule appears (fig. 13). The chromatin clumps now become granular and form an eccentric circle against the nuclear membrane, leaving an open center very much like the condition following the last division. The homogeneous granular border is at first deeply stained, but later loses its affinity for the haematoxylin and appears pale in color (figs. 11 and 16).

The idiochromosomes also seem to break up into unequal spherical bodies, three to eight in number, six being the more common number (fig. 16). In the clear central region the remains of the preceding spindle are quite apparent. Following this stage we have the reappearance of the idiochromosomes (fig. 17), and after that the entire nucleus appears granular, the central clear area disappearing and the two idiochromosomes stand out clearly (fig. 21).

It has not been possible to see anything like an unraveling stage as described by Wilson ('12) for stage c; the granular condition being directly followed by delicate threads (Wilson, stage d, fig. 22) which seem to push out and distort or break the nuclear wall. This is soon followed by the synizesis or contraction stage. Here the threads are drawn closely together and are located more to one side of the nucleus, the plasmosome and idiochromosome thread often remain outside of the contracted mass, as shown in figure 23.

Popoff ('08), Gates ('08), and Whiting ('17) look upon this as due to a rapid absorption of water by the nucleus; in other words, an osmotic effect; however, it has often been considered an artifact. Although at this stage of the growth period the spireme threads stain very intensely, making it difficult to trace the individual threads, it would look as though the filaments became arranged in the form of loops polarized with their free ends near the plasmosome and idiochromosome threads. Later on when the threads have thickened, this bouquet stage is much more clearly seen (fig. 25).

It has not been possible to see a side-by-side union of the spireme threads, the synapsis of Moore ('95), but the number

of filaments certainly is reduced and each one becomes much thicker. The threads now loosen up and occupy practically all the cell, the space between the nuclear membrane and the cell wall being quite small (fig. 24, Wilson ('12), stage f). I have not been able to find the longitudinal splitting of the thread —a process which Wilson ('12) describes as taking place.

There follows a period when it is hard to distinguish the threads as such (fig. 29, Wilson ('12), stage g). Wilson calls it a net-like arrangement. The actual breaking of the threads or pachytene stage is not well exemplified in Lepisma, but stage g is soon followed by the clumping of the chromatin into masses irregular in shape and joined together by linin threads (fig. 30). By a further condensation of these masses we get the prochromosomes. The formation of tetrads showing the quadrivalent condition of the autochromosomes is never apparent, neither is there any split indicating a parasynapsis.

The idiochromosomes retain their form and staining reaction until the formation of the delicate filaments (stage d), when they break up and form threads which are darker in color than the other threads, and one may be seen in close relation to a small plasmosome (fig. 35 a). The idiochromosome threads are at first very long and may extend across the entire width of the cell. They appear somewhat beaded, just as is the case with the threads of the autochromosomes.

During the later periods the threads show an end-to-end apposition, being joined by very fine linin fibers (fig. 35 m). The threads now become shorter and thicker assuming the U shape followed by the definite formation of loops with the plasmosome between them (figs. 20 and 35 j). Figures 35 i and h would seem to indicate that the limbs of the loop come together and become still more compact to form clumps lying against the nuclear membrane with the plasmosome still lying between them. For a considerable time the idiochromosome threads show a very clear inequality in that the thread nearest to the plasmosome is the longer (fig. 35 k).

A second small plasmosome may be formed and lies to one side with no attachment to either idiochromosome thread (fig.

35 e and f). Later I believe it fuses with the first, for the latter is seen to increase considerably in size and to show at times a double nature (fig. 35 g and k).

A third body similar in shape and staining reaction to that seen in the spermatogonia becomes quite prominent at this time (figs. 29 and 30), due to a slight increase in size and to the appearance of a clear transparent area encircling it. Painter ('14) describes in spiders similar small dark-staining spherical bodies, which he calls planosomes and which first make their appearance in the late spireme stage and which he was able to trace through the succeeding divisions. The planosomes, according to him, have spindle fibers, and would therefore be comparable to chromosomes, although as a rule they do not divide, but linger near the middle of the spindle and later go to one side.

From his description and figures, this body is the same as the one found in Lepisma domestica, only I find it first in the resting stages of the spermatogonia, and have not been able to follow it beyond the prophase stage of the first maturation division.

The first spermatocyte

With the condensation of the chromatin segments into the prochromosomes, the nuclear membrane breaks down and two chromosomes located near the periphery are seen joined together by a more or less ribbon-like connection, forming a V-shaped structure. Within or near the arms of the V the plasmosome may be found (figs. 36 and 37). With the exception of the prophase figures in which the idiochromosomes stain more deeply, there is no essential difference in the staining reaction of the idiochromosomes and the autochromosomes; but to make the behavior of the idiochromosomes plain throughout the different stages of the first maturation division, they have been drawn in black, while only the outlines of the autochromosomes are shown (figs. 36, 37, 41, 42, 43, 44, and 45).

The chromosomes arrange themselves on the spindle and in the metaphase plate (figs. 38, 39, and 40), the sex or idiochromosomes are still connected and one pair of the chromosomes is a

little further beyond the metaphase plate, so that in plate view one pair of chromosomes can be seen to be at a different level (figs. 39 and 40). The side view shows how one limb of the V extends farther than the other.

The metaphase plate (fig. 38), in which the idiochromosomes are located in the center and surrounded by a ring of chromosomes, reminds one of the arrangement in some Hemiptera.

There is little change in the position of these joined chromosomes in the anaphase (fig. 41), except for a shortening of the connecting thread and possibly a slight movement of the whole toward the distal pole. Figures 42, 43, and 46 picture the telophase arrangement, the idiochromosomes going undivided to one pole.

There are sixteen chromosomes plus the two idiochromosomes, or eighteen in all, in the first spermatocyte division. Side views have not been counted, owing to the great overlapping of the chromosomes. The plasmosome may be identified during the late prophase (fig. 37), but not definitely after the actual spindle formation. Bodies which are plainly not chromosomes are often seen in relation to the spindle, as the two equal bodies in figure 40, but whether these represent the divided plasmosome or are mitochondrial is not conclusive.

Resting stage of second spermatocyte

In the telophase of the first or early prophase of the second spermatocyte (fig. 46), the chromosomes are breaking up. Some appear unchanged, while others have swollen to a spherical shape and stain more diffusely. It is not possible to identify the idiochromosomes at this time, but a little later, when the resting nuclear stage is reached, the double nature of the idiochromosomes is quite apparent as the nucleolus in one of the now divided cells (figs. 47, 48, 50, and 51). It is not possible to confuse these resting second spermatocytes with the early spermatids, because both nuclear and cell size is much larger. The relative sizes of first and second spermatocytes and spermatids are shown in figure 33, which was diagrammed from measurements of the length

and breadth of ten representative cells of each kind, and the average diameter of each cell and of the ten cells taken.

In the second place, the chromatic nucleolus is distinctly double (fig. 47), while in the spermatid it is single and smaller (figs. 64, 66, and 68).

During the growth and division period, spindle remains stand out quite clearly as one, more usually as two vesicles, formed probably from the central fibers and showing a granular condensation in their interior (figs. 25 and 46).

The formation of the resting stage and the subsequent prophase is a rapid one, as I have observed resting nuclei, prophase, and dividing second spermatocytes in the same cyst. Figures 50 and 51, resting and prophase stages, respectively, are from a slide not particularly well fixed, as the cells are somewhat swollen, but figure 51 is interesting in that it shows the formation of spindle fibers before the nuclear wall has broken down and in figure 50 the idiochromosomes still show their double structure. The resting nucleus, at first granular, breaks up into faintly staining irregular or fantastically shaped entities without any visible unraveling stage and condense quickly into the prochromosomes (figs. 49 and 50).

The second maturation division

With the formation of the spindle for the second maturation division, two types of metaphase plates are seen: one (fig. 53) with eighteen chromosomes and another (fig. 56) with sixteen. In the latter case I have one perfect anaphase (fig. 59), in which both plates can be counted and both show sixteen chromosomes.

It appears that the idiochromosomes are now equal in size and no longer show a connecting thread. In the first maturation division the idiochromosomes were distinctly unequal, but each tapered into a thread connecting it with the other. This thread often seemed ribbon-like, granular, and taking the iron haematoxylin stain like the chromosomes.

It seems to attain its maximum length at the metaphase of the first maturation division and to shorten a great deal by the

time the telophase is reached, and it would appear as though this thread were fused with the smaller idiochromosome so that they both appear equal in the metaphase of the second maturation division. Another factor in favor of this hypothesis is that the chromatic nucleolus of the resting stage shows a double structure with hardly any inequality.

In the early anaphase (fig. 55) all the chromosomes show a longitudinal split near their centers, except two which represent, I believe, the divided idiochromosomes. The anaphase often shows the chromosomes arranged in the form of a ring (fig. 62). In figure 63 the chromosomes are at the poles and are beginning to form a nuclear membrane, but no change has taken place in the centrosomes. Figure 60, a late telophase, shows that one chromosome differs from the rest in being elliptical, while the others are V- or U-shaped and slender. A still later telophase is figured in figure 61, the chromatin now being massed at the poles. Two types of spermatids are formed, those with sixteen and eighteen chromosomes, respectively.

The centrosome in the spermatogonial and maturation divisions

In the archoplasmic mass or sphere representing the remains of the previous spindle one may occasionally see two dark granules (fig. 18), which I take to be the divided centrosomes. In the division figures of the spermatogonia centrosomes are difficult of demonstration, but in a few slides I can make them out as definite single granules at the poles of the spindle (fig. 12). I have never seen astral rays or anything comparable to a centrosphere at the time of division, but during the resting stages the centrosome is found in a granular sphere.

From the division figure of the last spermatogonial mitosis until shortly before the synaptic or contraction stage, the centrosome has not been traced, and when it does appear a considerable metamorphosis has taken place. At about the time when the fine spireme threads are being changed into loops, a granular mass can be made out at one end of the cell, and in this mass appear two short, stubby rods lying parallel to each other.

Later (figs. 20 and 26) the rods lengthen and show small granules at their ends. At first the two rods form an angle of 180°, but this angle is later decreased to 90° or less. Each rod now divides, but the halves remain attached by their granule ends, forming a pair of V-shaped centrosomes, each V representing a divided centrosome. This whole process is a rapid one, for all stages as well as the separation of the V's for some distance may be seen in cells which show little change otherwise. The migration is about completed and the V's nearly at the poles by the time the prophase condition is reached (fig. 30). During the succeeding division the apex of the V is directed toward the chromosomes, while its limbs touch the surface of the cell. The V may open considerably, nearly to a straight angle, so that a large part of the outer surface of the rods is in contact with the cell wall. The cells may also show a slight depression at the poles (fig. 39).

The spindle fibers all lead to the centrosome region, but an actual attachment of the fibers to the centrosomes, while taken for granted, does not show clearly in sections.

This V arrangement can be identified up to a late telophase of the primary spermatocyte, but I have not traced it through the resting stage of the second spermatocyte. Each second spermatocyte would receive one V, but when the rods reappear in the division figure they are divided, a single rod at either pole lying against the inner surface of the cell wall and oriented parallel to each other, but at a slight angle with the cell axis. The division or separation of the V's as well as their migration to opposite poles must take place during the resting period.

The centrosome rod can be traced through every succeeding stage to the early spermatid, where it may be seen lying free in the cytoplasm (fig. 65). In exceptional cases, as in figure 54, the rods have granules at their ends, or we may find a number of granules or fragments and no rod, as in figure 58.

The spermatid

The young spermatid cell is considerably smaller than the resting stage of the second spermatocyte. The chromosomes clump together, form a nuclear membrane, and quickly break

up. The nucleus appears round in polar view, but oval if looked at from the side. Later the nucleus becomes spherical, the chromatin appearing finely granular and congregated at the boundaries of the nucleus leaving an open center (fig. 66). One-half the cells show an idiochromosome nucleolus which usually presents a spherical part extending into the nuclear cavity and a flattened area against the inner surface of the nuclear membrane, while the other cells do not possess an idiochromosome nucleolus.

The methods of fixation and staining have a great deal to do with the structures observed in the spermatid. When strong Flemming is used for fixing followed by Heidenhain's iron haematoxylin, the cytoplasm of the early spermatid contains such a mass of intensely staining material that the nuclear membrane is made out only with difficulty. The same stain after Bouin's fluid brings out the nucleus and centrosomes, but not the cell inclusions.

At the very first, the cytoplasmic structures are somewhat loosely aggregated around the nucleus, but particularly between the nucleus and the last division plane. The centrosome can easily be followed from the telophase; located at first on the cell wall of the dividing second spermatocyte, it later moves inward, occupying the space between the cell wall and the nucleus (fig. 65).

As it moves around to get between the nebenkern and the nucleus, it turns 90° and comes to lie with one end on the nuclear membrane and the other against or near the cell wall (figs. 66 and 67). The rod-shaped centrosome now frequently shows a granule or enlargement at the nuclear end.

The nebenkern has meanwhile formed a broad ring of densely staining granular material in the center of which spindle remains of the last division appear and on either side two spherical bodies become visible (fig. 69), exactly like those seen in the two maturation divisions, and undoubtedly represent old spindles. In cross-section they appear as rings with their boundaries staining in varying degrees, often looking like crescents, and may possess a darker staining center. Looked at from the side, they take the form of rods with faintly stained material between them.

When the centrosome is in contact with the nuclear wall, one usually sees a granule at its inner end (fig. 66), and later a granule similar in size located near it on the membrane (fig. 67). This suggests the breaking away or division of the granule at the base of the centrosome.

The centrosome may now change its position, being found in the region of the nebenkern or even at the opposite side, and shortly the delicate axial filament is seen pushing from the cell and occasionally carrying a small clump of cytoplasm with it, very much as has been described by Buder ('15) in the Lepidoptera and called by him 'plasmaklumpchen.'

The single granule arising from the centrosome increases considerably in size and divides, giving rise to two granules which move apart and come to lie against the nuclear membrane and closely applied to it (figs. 65, 70, 71, 72, and 73). About this time or a little later a somewhat larger, round body condenses out of the nebenkern ring, as shown in figure 72.

Outside of the breaking up of the idiochromosomes and a slight tendency to become pale and homogeneous, the nucleus remains the same during the above changes in the cytoplasmic inclusions. In the stage which follows, the delicate axial filament is quite obvious and its outgrowth from the distal end of the rod centrosome is very clear. The rod has swung so that now it is in contact with the nucleus throughout its entire length and the thread is seen traversing the space between nucleus and cell wall (fig. 74). The nucleus contains numerous dark-staining granules. The spindle remains are prominent, their borders have increased in thickness, and now appear as irregular-shaped thick-walled vesicles. The nebenkern ring, cleared of the spindle remains and of the various granules as well as of several aggregations of granular mitochondria, now rounds itself up into an oval-shaped dense mass which later becomes round (fig. 76). No structure is at first apparent except a heavily stained granular body, but one soon sees a vacuolization of its border and we get the rosette nebenkern of many writers (fig. 74).

Of the three granules already mentioned, those arising from the centrosome have either disappeared or have become so

closely adherent to the nuclear membrane as to seem a part of it, while on the other hand the other body appears slightly larger (figs. 77 and 78).

The nucleus continues to stain darker, due to the enlargement of the chromatin granules, and these may become joined to each other and give the appearance of short threads (fig. 75). The vacuolization of the nebenkern continues at the expense of the central body which becomes smaller. The walls of the peripheral vacuoles break down, the spaces becoming larger and larger, until there is but one vacuole, which may exceed even the nucleus in size, with a small heavily staining central part (figs. 80 and 81).

From this period on, the axial filament is in close relation to the central body of the nebenkern, which in well-fixed material is now seen to be made up of a spireme-like thread. I have been able to follow it throughout the greater part of its course and I feel almost certain that it is a single continuous thread (fig. 80). The cell now begins to lengthen somewhat and the central part of the nucleus to stain heavily, the chromatin moving toward the nuclear center, leaving a clear transparent border (figs. 78 to 83).

Unless one is fortunate with his fixation and staining, the central part of the nebenkern shows no structure, but appears as a glassy elliptical body suspended in the single large vacuole by means of the axial thread, but it can be seen very clearly that the tail filament never enters the central body, but comes to lie against it. The vacuole membrane lengthens out as it increases in size, while the central thread-like structure breaks up into several large and many small vesicles (figs. 81 and 82). There are also mitochondria-like structures located between the nebenkern and the nucleus as well as some distal to the nebenkern.

The nebenkern membrane forms apparently the sheath of the axial thread, some cytoplasm forming clumps around the distal part of the thread, but the vesicles in large numbers fill the spaces between the spermatozoa as they increase in length. The middle-piece anlage enlarges, and by a turning of the nucleus, the axial filament comes to lie against it (figs. 85, 86, 88, and 90).

The body then flattens out against the nucleus and later elongates slightly (figs. 85 and 90). The nucleus lengthens, and as it does, the axial filament between the middlepiece and the centrosome does likewise. The centrosome, however, is now at the apex of the nucleus and will hereafter be considered as the acrosome.

At the time when the nebenkern membrane and its vesicles have completely broken up and are only apparent as end products ensheathing the elongated tails or located between the filaments, the nucleus is still spherical, compact, and does not take the haematoxylin stain very well (fig. 82). It still has a clear area about it and some mitochondrial material about the middle-piece anlage, which is a quite prominent body located usually on the opposite side of the nucleus from the acrosome. The axial filament arising from the acrosome at the apex of the nucleus is bent backward and passes near the middle-piece anlage.

The further changes are the loss of the clear ring about the nuclear chromatin by the spreading out of the chromatic material. The nucleus shows better staining qualities. The axial filament comes to lie nearer to the middle-piece and the latter may sometimes show one or more bubbles or vesicles, which are possibly mitochondrial, in relation to it (figs. 84, 85, and 86).

The nucleus now begins slowly to elongate and seems sometimes to pull away from the acrosome, so that part of the latter body may project beyond the nucleus. The nucleus continues to lengthen, the chromatin to appear paler in color. The middle-piece enlarges and elongates (figs. 88, 89, 90, and 91). The axial filament strand between the acrosome and the middle-piece remains applied against the nucleus and sometimes may show one or several splits in the thread, leaving an elliptical opening. In material stained for mitochondria, a cloud of granules seems to gather about the thread (fig. 87). Although it cannot be traced directly, I am of the opinion that this axial filament, which is loosely applied to the outer nuclear surface, becomes the undulating membrane of the mature spermatozoon.

Thompson ('17, p. 267) suggests the formation of the undulating membrane from a free flagellum in the Trypanosomes, as follows: "It is a plausible assumption to suppose that, as the flagellum waves about it comes to lie near and parallel to the body of the cell, and that the frill or undulating membrane is formed by the clear fluid protoplasm of the surface layer springing up in a film to run up and along the flagellum, just as a soap-film would be formed in similar circumstances." Of course the axial filament in this case is located between the nucleus and the outer cell wall, but it seems a reasonable hypothesis to think of the axial filament as having become loose from the nucleus and as able to draw out the thin layer of cytoplasm some little distance from the nucleus forming the undulating membrane.

The nucleus and middle-piece now become drawn out to considerable length, the acrosome decreases in size and we see a slight projection of the nucleus extending beyond the acrosome. The elongated nucleus stains darker and darker until no structure can be made out. From this point until the mature spermatozoa are reached I have not been able to make observations (figs. 93 and 94).

The spermatozoa

A study of the mature spermatozoa has been made by teasing the contents of the seminal vesicle in a minute quantity of physiological salt solution and either studying them alive in the solution or fixing the teased material in osmic acid fumes, hot corrosive sublimate, Bouin or strong Flemming, and staining.

The unstained living contents make an interesting study when examined by means of the dark-field microscope. In addition to the spermatozoa with their waves of movement extending from anterior to posterior end of the undulation membrane, there are a large number of small elliptical bodies performing active brownian movement. I thought at first that these bodies were the true spermatozoa and the others giant spermatozoa, but further study convinced me that this was not the case, for no tails could be found upon the small bodies, they stained only by plasma stains, and furthermore no stages in their development could be made out.

Occasionally the lumen of the vas deferens is partially filled with granules differing in size, and as the cells lining the vas deferens may show similar granules in their cytoplasm, I have considered the granular material of the lumen to be secretion products of the cells. Although the bodies present in the seminal vesicle are a little longer than broad and show a little difference in their size relations, yet I think they represent the secretion found in the vas deferens. Munson ('06) considers the epithelial cells of the vas deferens of the butterfly Papilio to have a secretory function, but unfortunately he does not figure or describe the process.

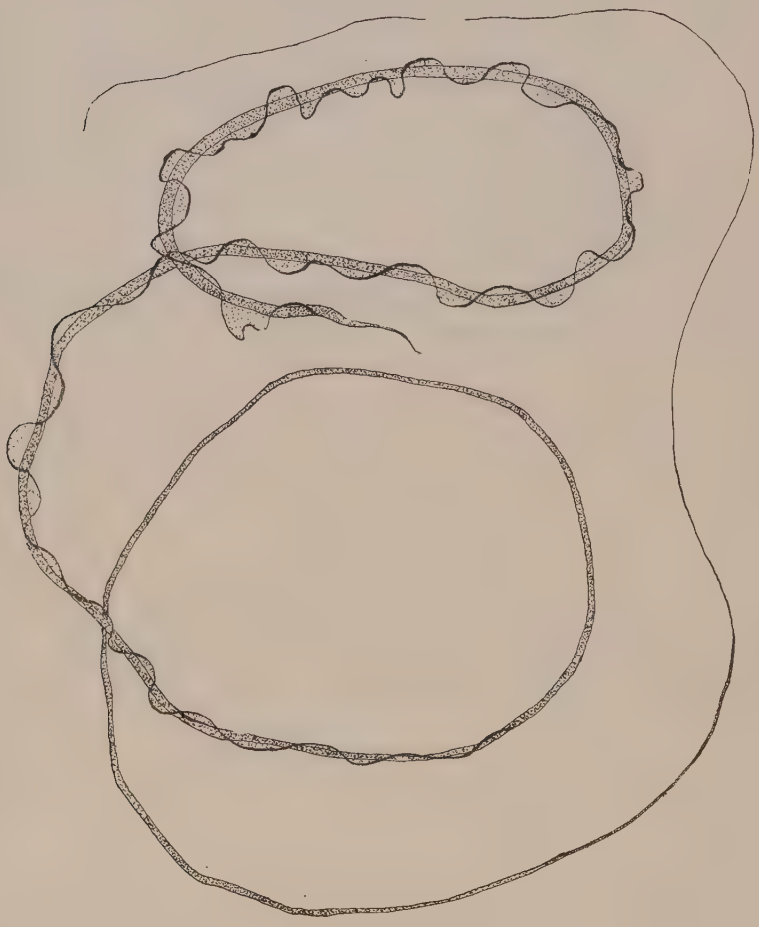
An added fact of interest is that the bodies are transmitted during copulation and are found in the seminal receptaculum of the female, which suggests that they have some function yet unknown to us.

To ascertain their nature, smears of the seminal vesicle were made, fixed in hot corrosive sublimate, and stained in orcein, safranin and orange G, Delafield's haematoxylin and orange G, safranin and malachite green, safranin and bleu de Lyon, Delafield's haematoxylin and erythrosin. In every case it was found that the granules took only the cytoplasmic stains. In order to test the possibility of the granules' being of a fatty nature, fresh smears were stained in sudan III, but the result was negative. Smears treated with ether showed also no effect of the latter on the granules.

When the spermatozoa are examined in the fixed and stained condition (text fig. A and fig. 95), one finds a long chromatin staining thread ending in a transparent fine point, the acrosome having disappeared, and extending from near the apex a conspicuous undulating membrane. The free edge seems formed of a little denser material and represents in all probability the proximal part of the axial filament, i.e., that part between the acrosome and the middle-piece.

It is almost impossible to see just where this membrane leaves off distally as it gets narrower gradually, but I should say that about the anterior two-thirds of the spermatozoon is provided with the membrane. It is not possible to find any trace of the

middle-piece or to see where the nucleus leaves off and the tail filament begins, as the latter structure becomes finer and more transparent until it is almost impossible to see where it ends.



Text fig. A Mature spermatozoon of Lepisma domestica arranged from successive camera-lucida drawings. × 2800.

I have noted a tendency for the tails of the living spermatozoa to stick together. The spermatozoa are very long, measuring from 400 to 660μ.

Mitochondria

Mitochondrial structures are present in the spermatogonia, but in very small numbers, and it is difficult to make them out. At the beginning of the growth period they appear clearly as a dark-staining crescent-shaped mass usually located at one end of the cell. This mass soon breaks up and forms some six to eight bodies differing from each other in shape and size (fig. 21), but retaining an almost constant number. These bodies take the stain intensely and appear as the most prominent structures during the entire growth period. As the cell increases in size, the mitochondria becomes so conspicuous, even by ordinary iron-haematoxylin staining, that the cytoplasm seems like a dark border about the nucleus, and in this dark-staining mitochondrial matrix the clumps stand out clearly.

During the first maturation division the ring of granular mitochondria encircles the entire spindle, while the larger bodies are scattered about the cytoplasm and are located near but not on the spindle. The mitochondrial material seems to divide equally, half the granular material as well as four clumps going to each cell (fig. 39). In the resting stage of the second spermatocyte the mitochondria forms a narrow dark-staining ring about the nucleus, but the clumps are no longer apparent (figs. 49, 50, and 51). In figure 54 the granular mass is seen arranged about the spindle of the second division, while figure 61 indicates how they gather about the chromatin at the poles of the young spermatids. In the description of the spermatids the further history of the mitochondria has already been given.

DISCUSSION

The resting stage

While in many animals no resting nucleus is formed following the first maturation division, the chromosomes of the telophase being quickly transformed into the prophases of the second division, there are quite a number of exceptions reported in the literature.

Murray ('98) finds a well-marked resting nucleus in the Pulmonates, Helix and Arion. McGill ('04) found it to happen occasionally in the dragon-flies, and Painter ('14) describes it as occurring in the spiders with the accessory chromosome persisting as a nucleolus. Kingsbury ('01) finds in the salamander Desmognathus fusca, that a nuclear membrane is formed following the first maturation division, but that the chromosomes never lose their individuality.

In Lepisma domestica the chromosomes, with the exception of the idiochromosomes, entirely break up and a nuclear membrane is formed. While it is undoubtedly of short duration, still the outward individuality of the autochromosomes is lost and the second division is preceded by their reformation.

The idiochromosomes

Wilson ('09) divides the sexual differences of the chromosomes into five and possibly seven types. Lepisma domestica falls in line with his type IV in which "the male has a pair of idiochromosomes, half the spermatozoa receiving both and hence two more than the other half."

Only one form has been found which has this arrangement, the coreid species Syromastes marginalis L. This form was first described by Gross ('04) and again by Wilson ('09). The accessory chromosome arises by a synapsis of two spermatogonial chromosomes which divide equationally in the first spermatocyte, but fail to divide in the second.

Lepisma domestica differs in that the two spermatogonial chromosomes do not fuse, but remain separate and joined by a stout thread. They pass undivided to one pole in the first spermatocyte division, but separate in the second. Wilson's prediction that the female of Syromastes would have two more chromosomes than the male, he afterward found to be the case. Reasoning in a similar manner, Lepisma domestica females should have thirty-six chromosomes, but unfortunately I have been unable to make any chromosome counts so far in the female.

Synapsis and reduction

It has not been possible in the ordinary chromosomes or autochromosomes to see whether there is either a side-by-side union of the spireme threads, a parasynapsis, or an end-to-end conjugation, a telosynapsis. It is clear, however, that the spireme threads in postsynaptic stages are much thicker and are present in fewer numbers. Whether they are half the leptotene number or not could not be made out.

In the case of the idiochromosomes the conclusions are clearer. Each idiochromosome breaks up into a spireme thread and the two threads eventually unite end to end, one of them being attached to a large plasmosome. From these threads two chromosomes are formed by the condensation of the chromatin, but they still remain united by a thread which is probably linin in nature and along which, when the thread lengthens, the chromatin is drawn out.

Synapsis, or a side-by-side conjugation, if it takes place at all, does so following the telophase of the first maturation division. That the idiochromosomes do come into a very close relation is shown by the longitudinal split apparent in the idichromosome nucleolus of the resting nuclei of the second spermatocyte (figs. 47 and 50).

If, as is generally conceded, the spermatogonial chromosomes represent two groups, one of maternal and the other of paternal chromosomes, and the homologous pairs conjugate at synapsis then each of the idiochromosomes represents one spermatogonial chromosome. A further proof of this is that the thirty-four spermatogonial chromosomes, judged from their size, are all of the same valence, i.e., bivalent. After synapsis the autochromosomes are quadrivalent, but definite four-part tetrads are not apparent during the prophase, and at the metaphase the chromosomes are dumb-bell-shaped, the longitudinal pairing leaving no trace. However, in one cell I have found the idiochromosomes at the time of anaphase showing a bivalent construction (fig. 45).

The first maturation division separates the dumb-bell-shaped chromosomes transversely (fig. 39), and probably represents a reduction division, as the idiochromosomes go to one pole undivided. The second division of the autochromosomes is clearly a longitudinal one (fig. 55), while the idiochromosomes separate transversely, so that it would seem that this represents an equational division of the autochromosomes and the separation of the idiochromosomes one from the other.

The centrosome

The single- or double-rod type of centrosome has been described in a variety of forms. Meves ('98) and Buder ('15) have described them in the Lepidoptera, and Sewertzoff (Meves, '00) in Orthoptera, and Korff ('01) in the Coleoptera. In plants Von Mottier ('98) found them in the tetraspore mother cell of Dictyota dichotoma. Korff also found them in the sperm cells of the domestic hen and duck, while Hortega ('16) figures them for the ganglion cells and brain of man.

Von Mottier does not consider them homogeneous, but to arise from small granules. In the beetles, Korff shows them to be very like those found in Lepisma domestica, but he has not reported any granule in relation to the rods at any time. He finds the limbs of the V separating in the late telophase and appearing parallel to the polar axis in the spindle. In Lepisma domestica the centrosomes are always oblique to the axis, but parallel to each other.

We have seen the centrosome first as one or two small granules, then as double rods with or without end granules, and still later as single rods which only occasionally show a granule. In very rare cases, instead of single rods, the centrosome consists of several granules in the polar position. From these observations the form of the centrosome would seem to be a variable quantity. It is interesting in this regard that Korff ('01) was only able to see the V-shaped centrosome in the sperm cells of the drake and rooster, while all the other cells of the body showed centrosomes consisting of single granules.

The acrosome

Although a number of the older writers, notably Platner ('89), Niessing ('96), Field ('95), and Moore (94'), have described the acrosome as arising from the centrosome, Wilson ('06) comes to the conclusion that the work of Henking ('91), Wilcox ('96), and Paulmier ('99) show conclusively that in the insects the acrosome is derived from the nebenkern.

That this is not the case in Lepisma domestica can be easily proved, for in every stage from the telophase of the second division until the oldest transformation stage in which it was possible to identify structures, the centrosome rod and its change into the acrosome can be followed.

It might perhaps be argued that the granule is really the centrosome and the rod only a product of the centrosome, formed in somewhat the same manner as the 'battonet' or rodlet in the spermatid of the Pribilof fur seal. As described by Oliver ('13), it arises as a prolongation from the anterior centrosome. In any event, the acrosome owes its origin either directly or indirectly to the centrosome.

Goldsmith ('19) describes in the tiger-beetle a condition, which in view of my own work on the acrosome and middle-piece, is very suggestive. He figures an extra nuclear plate or middle-piece which is formed at the point of junction of the axial filament and the nucleus. It contains several chromatin-staining bodies to which the axial filament is attached. These chromatin bodies move to one side and then toward the anterior end of the nucleus, the filament coming at last to lie against the elongated mitochondrial body (nebenkern) and the bodies to assume a bivalent appearance at the anterior end of the nucleus. The middle-piece becomes drawn out into a granular thread continuous with the axial filament, while the acrosome appears later and fuses with the other two bodies.

It would appear that the chromatin-staining bodies, to which the axial filament is attached, must be the centrosomes, and that their change in position, due to the rotation of the nucleus, is exactly parallel with what occurs in Lepisma domestica.

While no opinion as to the origin of the acrosome is advanced, the fact that it arises in relation to the granules from which the axial filament originally developed would point to the probability of a centrosomal origin. The granular thread which he considers to be the drawn-out middle-piece is, in Lepisma domestica, simply the proximal end of the axial filament carried to the apex of the cell along with the centrosome, and it would seem as though such an interpretation could be made of Goldsmith's results.

The middle-piece

Wilson ('06, p. 337), in speaking of the essential structures in a spermatozoon, lists the middle-piece as a body which "either contains a formed centrosome or a pair of centrosomes, or is itself a metamorphosed centrosome."

It is altogether possible that the middle-piece in Lepisma domestica arises from one or both of the granules which we found to have their origin from the centrosome and later traced them to where they were closely applied to the nuclear wall. In fact, we would naturally expect something of the kind, but unfortunately the later history of the granules could not be followed. The question is further confused by the presence of a body which condenses from the nebenkern ring and which, from its size, staining power, and position, would point to its becoming the middle-piece. Furthermore this body can be found practically in all the stages up to the apposition of the middle-piece to the nucleus, but here again we are stopped, for we have not seen the actual formation of this body into the middle-piece. It is possible that a further study will solve this difficulty.

Comparison with Orthoptera

At the first glance there seems to be a similarity between the spermatogenesis in Thysanura as described above and that in Orthoptera as previously described and particularly given by Payne ('16) for Gryllotalpa borealis, but there are also differences which cause one to question whether the resemblance is not more superficial than real.

In the first place, the general shape and arrangement of the chromosomes in the spermatogonia of Gryllotalpa borealis are very much like similar stages in Lepisma domestica. Payne also has described changes in the mitochondrial mass of the spermatid (his figures E, F, and G, pl. 2), which have almost exact counterparts in Lepisma domestica. Then again his figure J on plate 3 shows an axial filament in which the cytoplasm is so arranged in waves as to look like the undulating membrane found in the Thysanura.

A comparison of the group of chromosomes associated probably with sex in the two forms shows, however, several important differences. In Gryllotalpa borealis Payne finds a single chromosome which does not divide in the first maturation division and therefore could be directly compared with the idiochromosomes of the Thysanura were it not for the fact that the single chromosome is associated with an unequal bivalent chromosome and in division always goes to the same pole as the large 'end' of the unequal chromosome. Therefore, the two resulting secondary spermatocytes differ not only in that one has an extra chromosome, but also in that the same cell possesses the large 'end' of the unequal chromosome, while the smaller part passes to the other cell. Payne favors the view that these chromosomes represent a triad group rather than an unequal pair of idiochromosomes and an accessory chromosome.

Payne has not been able to trace the centrosome of the second maturation division through to the spermatid, and in fact has not been able to demonstrate the presence of the centrosome in the spermatid at all, although he presumes that the body from which the axial filament arises and which later becomes the middle-piece may be a centrosome. Further, he describes the acrosome as arising from an elongated body which suddenly appears de novo in the cytoplasm, whereas in Lepisma domestica the acrosome is formed from a rod-like centrosome.

SUMMARY

1. The male Lepisma domestica has three pairs of testes on each side, each testis connected by a duct with the respective vas deferens.

2. The blind end of the testis contains the youngest stages.

3. Primary spermatogonia are formed very early in life.

4. Thirty-four chromosomes are present in the spermatogonia. A chromatic nucleolus is present in the resting stage of the spermatogonia.

5. The growth stages follow the description given by Wilson ('12).

6. A planosome is seen in the resting stage of the spermatogonia appearing in the growth stages as a much larger body.

7. One or two plasmosomes appear shortly after formation of the spireme threads, and disappear later.

8. There are eighteen chromosomes in the first maturation division. The two idiochromosomes pass undivided to one pole.

9. The autochromosomes divide longitudinally in the second division, while the idiochromosomes do not. Instead, they separate, each spermatid receiving one idiochromosome.

10. The form of the centrosome is changeable, but its almost constant presence either in the shape of a granule or of a rod indicates that it may be a permanent cell structure.

11. A chromatic nucleolus is present in half of the spermatids.

12. The nebenkern is formed from granular mitochondria, the remains of the last and of two previous spindles.

13. The axial filament grows from the end of the rod-shaped centrosome which forms the acrosome.

14. Another body, presumably derived from the nebenkern, forms the middle-piece.

15. The nebenkern, after separating out the spindle remains and several accumulations of mitochondrial material, form a vacuolated body which furnishes a sheath for the axial filament.

16. The axial filament persists and forms the undulating membrane of the mature spermatozoa.

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EXPLANATION OF PLATES

All the figures, with the exception of the first three, were made with a Zeiss 2-mm. apochromat. objective and Zeiss compensating ocular no. 12. In order to get as high a magnification as possible, drawings were made at table level, giving an enlargement of about 1850 diameters.

ABBREVIATIONS

a, acrosome
af, axial filament
c, centrosome
i, idiochromosomes
m, mitochondria
mp, middle-piece
n, nebenkern
o, ovipositor
p, penis
pl, plasmosome
ps, planosome
s, spindle remains
sv, seminal vesicle
t, tubules
vd, vas deferens
x, middle-piece anlage

PLATE 1

EXPLANATION OF FIGURES

1 Posterior segments, ventral surface, of male Lepisma domestica. × 20.

2 Posterior segments, ventral surface. of female Lepisma domestica. × 20.

3 Testes of one side showing connection with vas deferens and seminal vesicle. × 20.

4 Spermatogonium, surface view, showing resting condition of nucleus and the attraction sphere.

5 Prophase of spermatogonium.

6 Metaphase plate early spermatogonium. Thirty-four chromosomes joined by linin threads.

7 Telophase spermatogonia showing persistent spindle remains.

8, 9, 10, and 11 Nuclei of spermatogonia decreasing in size with each division.

12 Spermatogonial metaphase from the side. Centrosome as single granule at poles.

13, 14, and 15 Resting stages spermatogonia to show idiochromosomes.

16 Spermatogonium showing the breaking up of the idiochromosomes.

17 Beginning of growth period. Idiochromosomes reformed.

18 Spindle remains from spermatogonium with two centrosome granules.

19 Same as the preceding from early growth period with V-shaped centrosomes.

20 Centrosome rods with granules at their ends, before division. Plasmosome and idiochromosome loops.

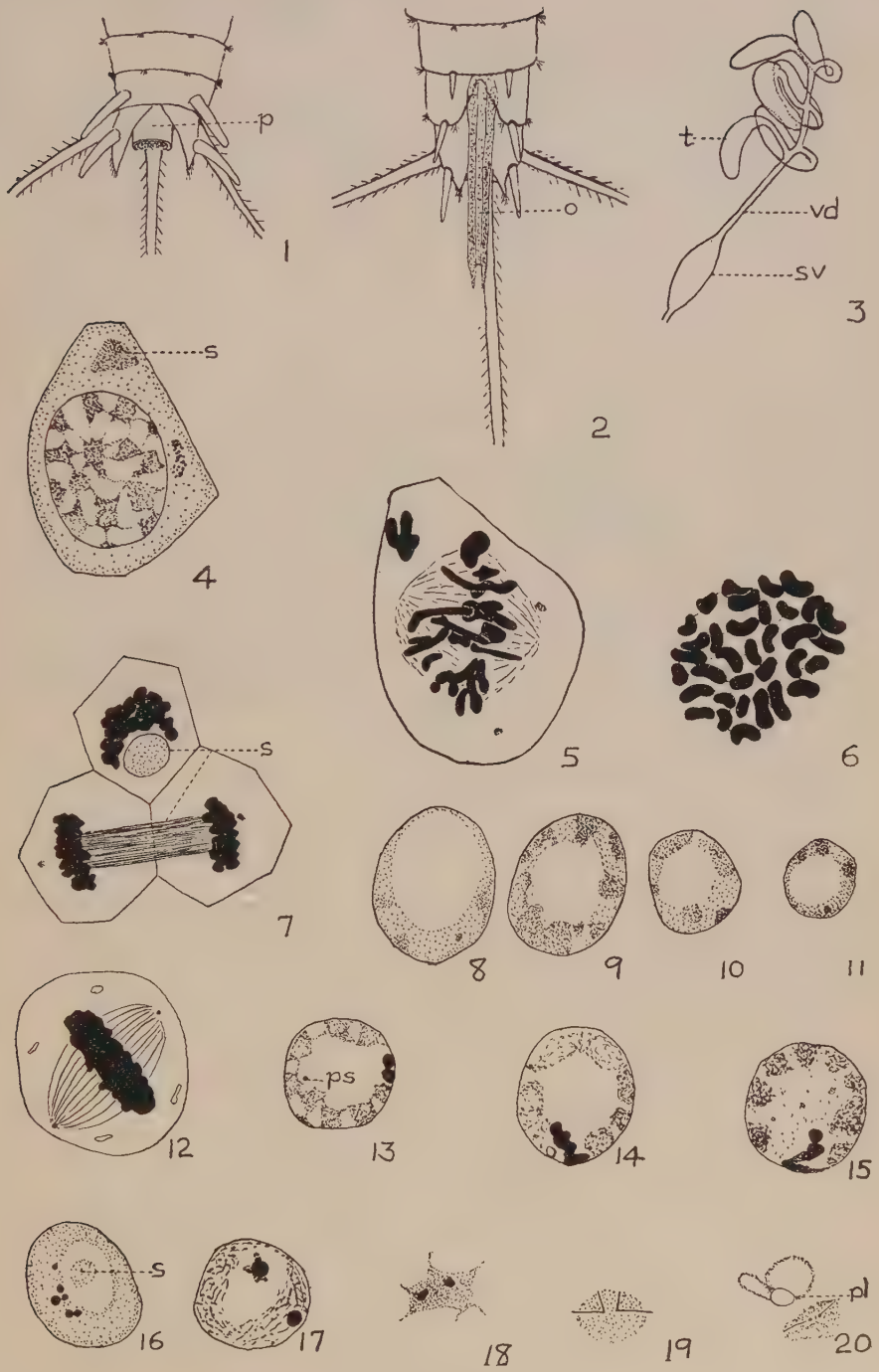
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PLATE 2

EXPLANATION OF FIGURES

21 First spermatocyte. Nucleus homogeneous except for idiochromosomes.

22 First spermatocyte showing fine, loosely arranged leptotene thread. Idiochromosomes very pale in color.

23 Early contraction stage of first spermatocyte. Plasmosomes with idiochromosome thread to one side.

24 First spermatocyte showing a spreading out of the threads following contraction. Plasmosome and idiochromosome threads shown in surface view.

25 Bouquet stage of first spermatocyte. Only a few loops shown in drawing.

26 Pachytene stage of first spermatocyte.

27 Same as the preceding, only somewhat later.

28, 31, and 34 Sertoli cells associated with different stages in the development of the spermatozoa.

29 Confused or net-like stage of first spermatocyte. Planosome prominent.

30 Early prophase of first spermatocyte. Centrosomes have migrated nearly to poles. Two plasmosomes present.

32 Dividing cell from spermatogonial region. Possibly a Sertoli cell. Chromosomes paired.

33 Diagram to show the relative sizes of cells of first and second maturation divisions and of the spermatid.

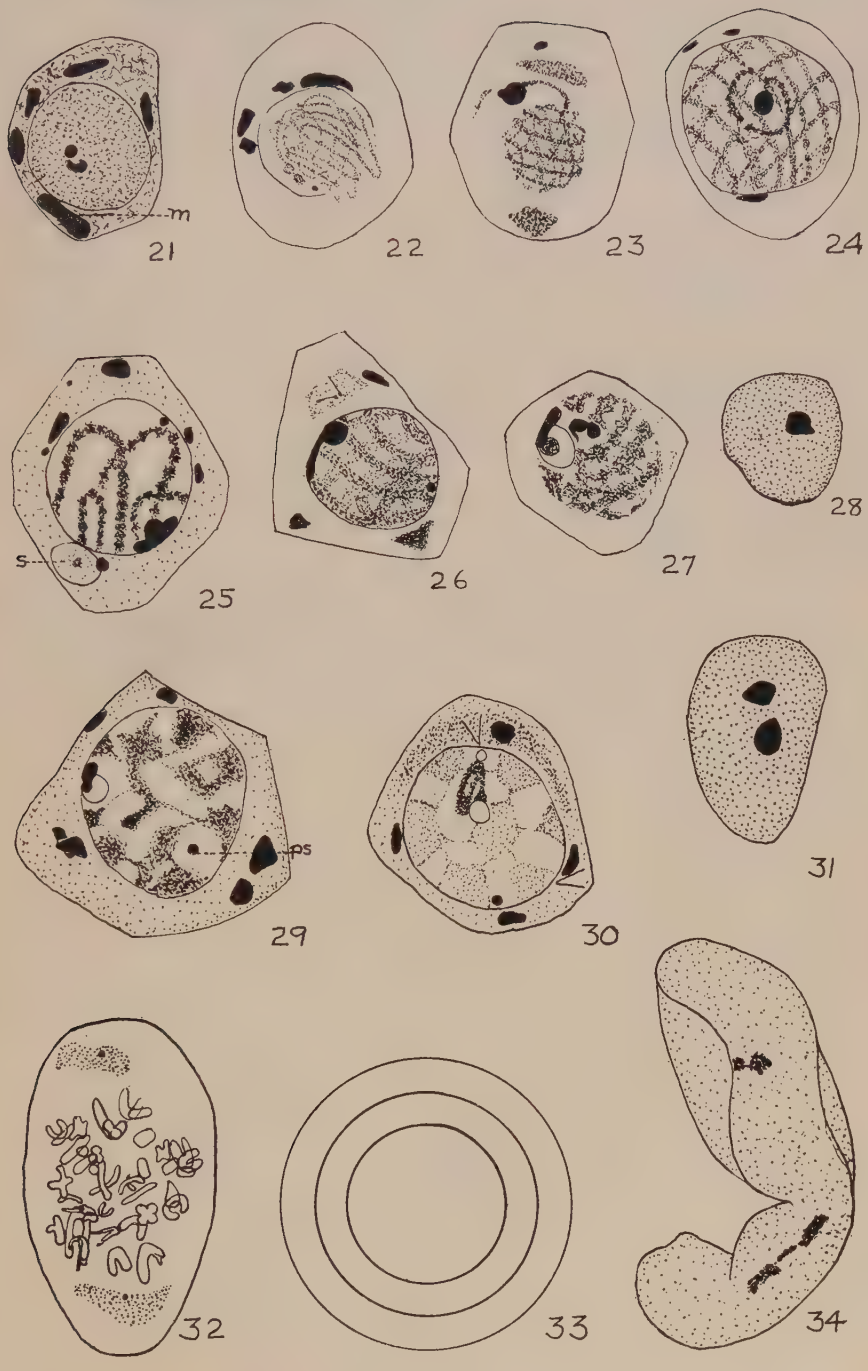
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PLATE 3

EXPLANATION OF FIGURES

35 The plasmosomes and idiochromosomes during the growth period of the first spermatocyte showing formation of spireme threads and their contraction into the prochromosomes.

36 and 37 Prophase of first spermatocyte showing connected idiochromosomes.

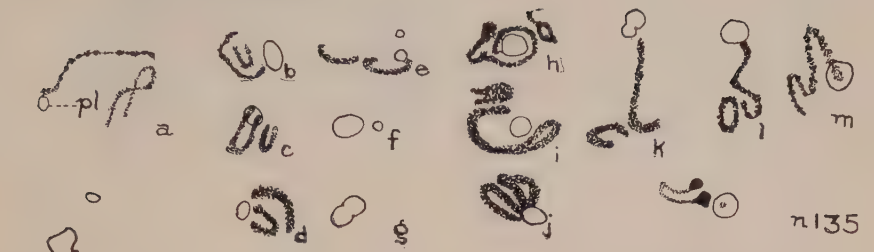
38 Metaphase plate of first spermatocyte with idiochromosomes in center.

39 Side view metaphase of first spermatocyte to show dumb-bell-shaped chromosomes and their transverse division. Idiochromosomes joined together.

40 Same as the preceding.

41, 42, 43, and 44 Side views of dividing first spermatocyte, showing idiochromosomes during anaphase and telophase.

45 Oblique view of anaphase of dividing first spermatocyte. Thirty-three chromosomes plus the idiochromosome complex which here shows each idiochromosome as bivalent.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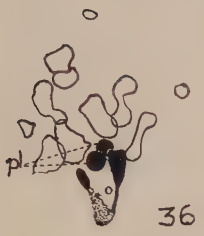
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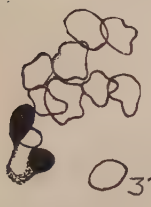
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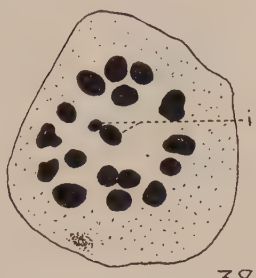
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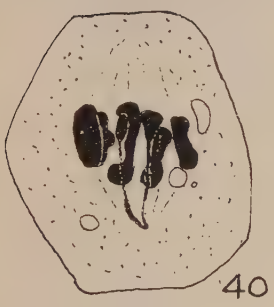
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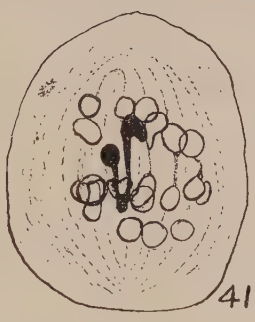
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PLATE 4

EXPLANATION OF FIGURES

46 Early second spermatocyte, showing the breaking up of the telophase chromosomes. Position of spindle remains typical.

47 Resting stage of second spermatocyte.

48 Same as the preceding, the chromatic nucleolus found in only half the cells of the second spermatocyte.

49 Early prophase of second spermatocyte.

50 Same as the preceding, chromatic nucleolus double. Ring of mitochondrial granules around the nucleus.

51 Resting stage of second spermatocyte, showing formation of spindle fibers before the prophase stage is reached.

52 Side view metaphase of second spermatocyte.

53 Eighteen chromosome metaphase plate of the second spermatocyte.

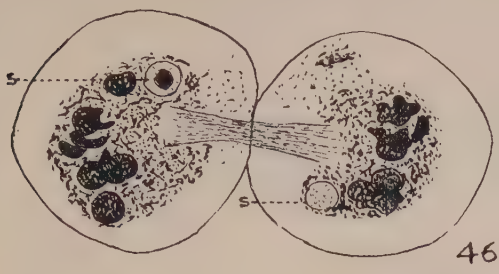
54 Side view metaphase plate of second spermatocyte. Rod centrosomes with granules at inner ends.

55 Early anaphase of second spermatocyte with the autochromosomes dividing longitudinally, while the idiochromosomes have separated transversely.

56 Metaphase plate of second spermatocyte. The sixteen chromosome type.

57 Later anaphase of second spermatocyte.

58 Same as the preceding with granules at poles instead of rods.



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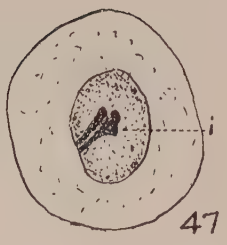

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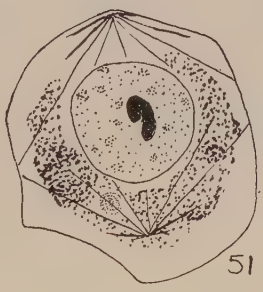
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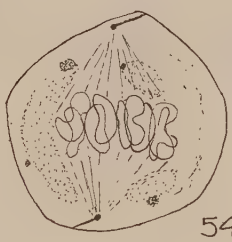
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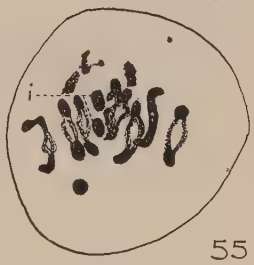

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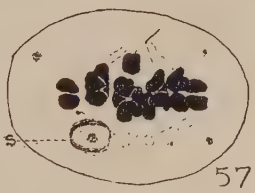

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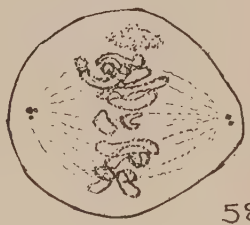
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PLATE 5

EXPLANATION OF FIGURES

59 Two anaphase drawings of second spermatocyte from same cell. Sixteen chromosomes in each.

60 Spermatids from division of eighteen chromosome second spermatocyte. The divided idiochromosomes differ in shape and are darker stained than the autochromosomes.

61 Young spermatids before the reorganization of nucleus.

62 Anaphase of second spermatocyte, showing ring-like arrangement of chromosomes.

63 Early formation of nucleus of spermatid.

64 Spermatid with resting nucleus and nucleolus.

65 Early spermatid with rod centrosome.

66 Same as the preceding, showing centrosome with granule at base lying against nuclear membrane.

67 Same as the preceding. The granule broken off.

68 Same as the preceding. The granule much enlarged.

69 Elements of nebenkern from early spermatid.

70 Spermatid showing division of granule.

71, 72, and 73 The nebenkern ring from spermatid, showing separation of granules and formation of new body from the nebenkern.

74 Central part of nebenkern of spermatid, showing rosette form and spindle remains.

75 Spermatid showing vacuolization of nebenkern border further advanced. Nuclear granules larger and stain darker.

76 Spermatid showing centrosome applied to nuclear membrane.

77 and 78 Spermatid showing clear space forming around chromatin of nucleus. Middle-piece anlage present.

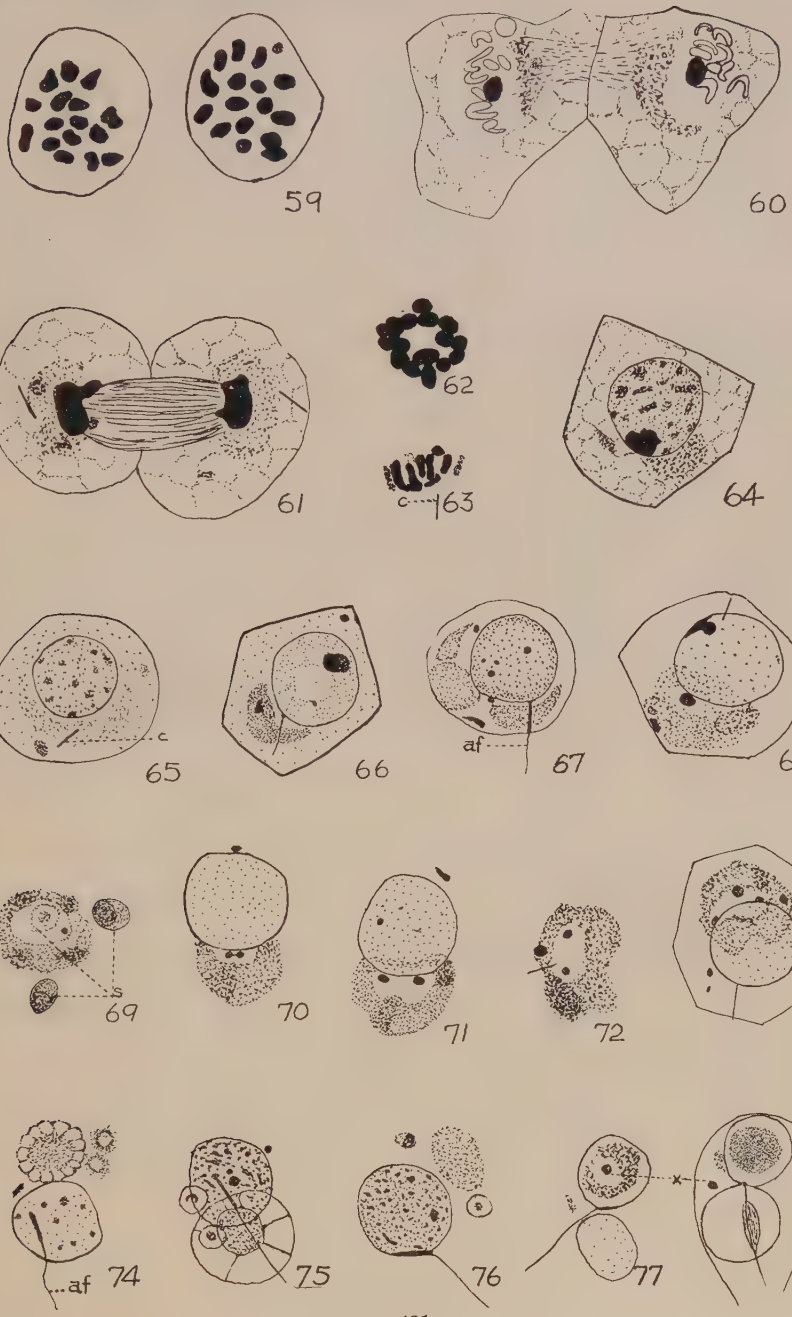
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PLATE 6

EXPLANATION OF FIGURES

79 Same stage as figure 78. Centrosome rod shown clearly.

80 Spermatid with chromatin of nucleus more condensed. Thread-like structure in middle of nebenkern vacuole. Mitochondria between nebenkern and nucleus as well as distal to nebenkern.

81 Same as the preceding, but threads of nebenkern have broken and now form vesicles.

82 Same as the preceding showing linear arrangement of small vesicles.

83 Spermatid of a slightly later state than the preceding.

84 Spermatid with nebenkern contents thrown off.

85 and 86 Spermatids. Side and surface view adjacent cells to show relation of axial filament to middle-piece anlage. Filament thicker on account of mitochondrial granules in relation to it.

87 Spermatid showing mitochondrial granular mass in relation to middle-piece and axial filament.

88 Spermatid showing secondary relationship to middle-piece anlage.

89 Spermatid. A later stage to show mitochondrial ribbon about the proximal part of the axial filament.

90 Spermatid nucleus elongating and staining darker. Middle-piece getting longer.

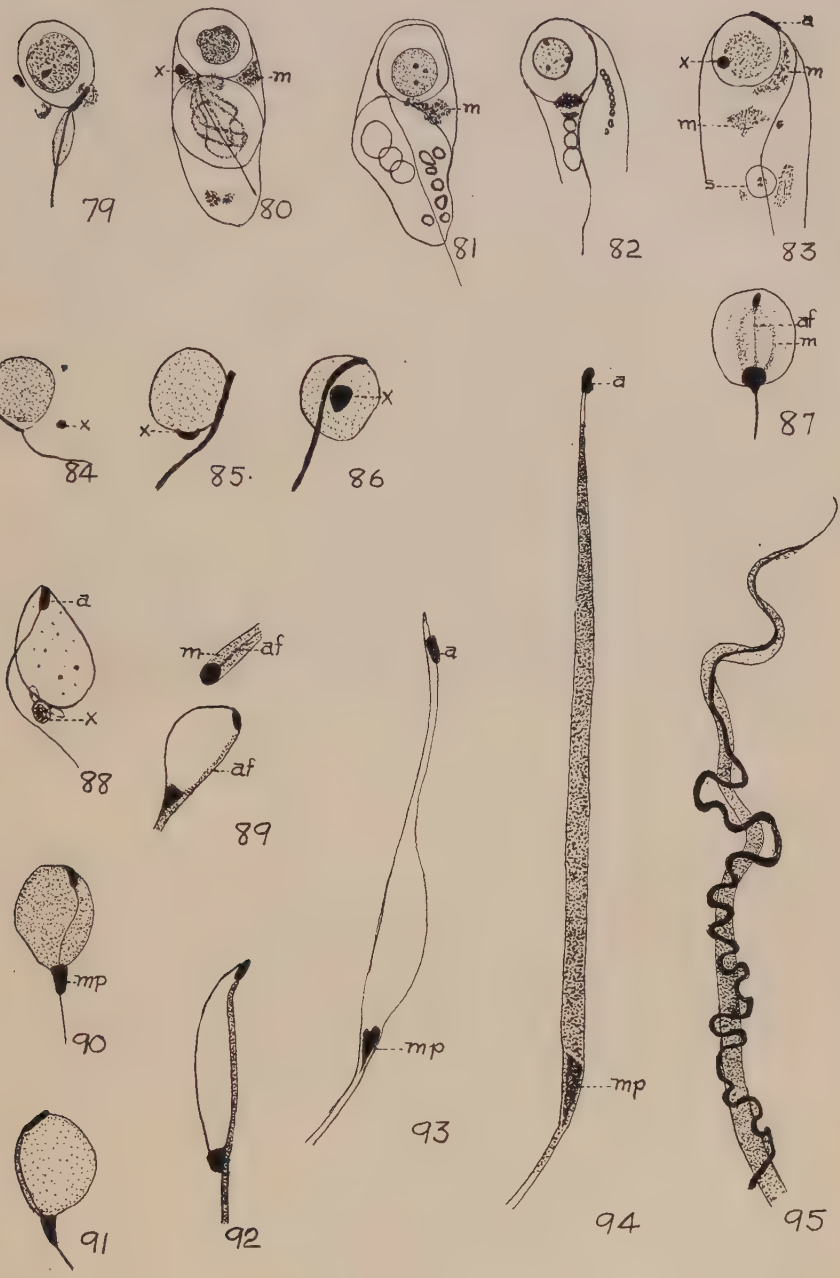
91 Same as the preceding.

92 Elongation of nucleus of spermatid. Mitochondria about filament.

93 Spermatid. A still later stage, no mitochondria shown.

94 Spermatid nucleus very much drawn out. Acrosome at apex and darker part indicates position of middle-piece.

95 Part of anterior end of mature spermatozoon. The undulating membrane with its thicker border is well shown.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EXPERIMENTS ON THE DEVELOPMENT OF THE GILLS IN THE AMPHIBIAN EMBRYO

[Reprinted from Biological Bulletin, Vol. XLI., No. 3, September, 1921.]

EXPERIMENTS ON THE DEVELOPMENT OF THE GILLS IN THE AMPHIBIAN EMBRYO.[1]

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The present work upon the gills was undertaken as a continuation of the study of relations of symmetry in embryonic organs, in the hope that it might throw light on questions raised in previous studies of the limbs.[2] The external gills of the salamander were chosen to this end, as constituting another system with right and left enantiomorphs, each without any plane, axis, or center of symmetry in itself. Owing to the complexity of the system, however, if for no other reason, the experiments failed to give as clear evidence on the questions raised as the limbs had done. Nevertheless, they have brought to light certain interesting facts, which in part corroborate and in part extend those reported by Ekman ('13), who worked upon various anuran embryos. They show, furthermore, certain differences between *Amblystoma* and the anurans with respect to the organs studied.

The gill arches and external gills form a complex in which all of the germ layers are represented. There is an outer covering of ectoderm, a central core of mesoderm, which contains the blood vessels and extends out into the gill filaments, and an inner lining to the arches and the clefts consisting of endoderm. Besides these the ganglion crest contributes elements to the arches, chiefly to the formation of the cartilaginous skeleton.

The experimental work of Ekman upon anurans has shown the following: (1) That when the ectoderm covering the gill region is removed, the regenerated ectoderm is capable of forming gill filaments. (2) That when the gill ectoderm is replaced by ectoderm from another region, with certain exceptions, the for-

[1] Read before the National Academy of Sciences, November 17, 1920.

[2] Harrison ('17 and '21).

mation of gills is suppressed. (3) That the external gills and the outer branchial grooves are specifically determined in the ectoderm and that the endoderm plays no part in the localization of the gills. (4) That the ectodermal rudiments of the gills have, from early stages on, a certain power of self-differentiation which varies amongst the several species studied. (5) That gills which arise from displaced ectoderm, together with atypical endoderm, conform entirely as to their position, form, and size, to the ectoderm. (6) That the branchial blood vessels are an essential factor in the later development of the gills.

The present experiments were made upon the embryo of a urodele, *Amblystoma punctatum*. They concern almost entirely the two outer layers, ectoderm and mesoderm. Owing to technical difficulties, the endoderm was experimented with only to a very limited extent, and this layer, therefore, does not figure in the present analysis. While certain things are quite definite, the work must be considered as incomplete, inasmuch as there has been no opportunity as yet to examine the specimens in sections.

With regard to the technique of operation, only one thing requires special mention. The donor embryo was in most cases stained *intra vitam* in Nile blue sulphate, which colors the ectoderm bright blue.[3] The stain persists for many days, so that the exact delimitation of the graft can be observed, as development proceeds. This removes a serious source of error, for it is thus always possible to determine whether the parts under observation are derived from the host or the graft. Embryos from stage 21 (Fig. 1, medullary folds just closed) to stage 25 (just before appearance of tail bud[4]) were used for nearly all the experiments. A few were made with earlier and a few with slightly later stages. Subsequent experiments made with still later stages show that marked changes in the behavior of the ectoderm after transplantation occur at about the time when the mesectoderm from the ganglion crest grows down and the first branchial pouch is developed (stage 29). These will not be considered at present.

[3] Detwiler ('17).

[4] Stages 25 and 29 have been figured previously (Harrison '18, Figs. 1 and 2).

Removal of the Ectoderm of the Branchial Region and its Replacement by Ectoderm from Another Part of the Body.

The gill region in an embryo with closed medullary folds is shown in outline in Fig. 1 (*BR*). If this ectoderm is removed and replaced by ectoderm from the flank (*FL*) or any other part except that immediately surrounding the gill region, complete suppression of the operculum and the gills on the operated side (Fig. 2) results. Thus, out of twenty-four cases in which ectoderm from the flank was used, seventeen had the gills entirely lacking. In six cases in which the third gill developed and in one in which the first gill was formed, ectoderm from the host covered the greater part of the gill including the tip.

When the covering ectoderm is taken from the region just behind the gills, *i.e.*, from the pronephric and limb area (*PN*), functional gills develop. This was found in five cases, in three of which all three gills were well developed, while in the two others the first gill was either absent or rudimentary.

When, however, the ectoderm is taken from the ventral mid-region (*CD*), *i.e.*, from the region immediately ventral to the branchial, the result is not so favorable. Out of nine cases, no gills at all developed in four, a functional third gill in one, a functional third and rudimentary second in three, and two functional gills (second and third) in one.

Likewise, when the covering is taken from the anterior part of the head, there is usually a partial development of the gills, involving principally the second and third. The transplanted piece was taken in various ways (Fig. 1, *C*, *C'*, *A*). In general, it may be said that the closer the seat of origin of the graft is to the normal branchial region, the more perfect are the gills that develop. A supernumerary balancer developed in almost all of the cases in which the graft included the normal balancer ectoderm.

When the ectoderm covering the gill region is removed and the wound left uncovered, as it was in the donor embryos in many experiments, the surrounding ectoderm pushes over the wound, covering it usually in twenty-four hours or a little more.

The mortality among these embryos is high, but when they live, gills nearly always develop from this regenerated ectoderm. In some cases the gills formed are normal; in others, there is a reduction in number or in size or both, the first gill being more often affected than the others.

Bilateral Extirpation of Branchial Ectoderm.

As far as the question of the morphology of the gills is concerned, this experiment is in no wise different from the unilateral operations, but physiologically it may prove of greater interest. At present only a few cases are available for study. In three experiments the branchial ectoderm was removed on both sides and replaced by ectoderm from the flank. In two of these cases no gills at all developed, while in one the first gill on the left side, which was partly covered by ectoderm from the host, was rudimentary. In the last-named case the circulation of the blood was never established and the embryo became œdematous. In both of the other cases the circulation was established in the vessels of the yolk, but the heart action became more and more feeble, and after a time the circulation of the blood ceased entirely. Both of these eventually became œdematous also, though not extremely so. One individual, killed fourteen days after the operation, showed marked lack of sensitiveness to tactile stimuli, and responded to a needle prick only with a single jerk. It was apparently unable to swim. The other could execute swimming movements fairly well, though in a somewhat jerky fashion. A fourth case that should be considered in this connection had the branchial ectoderm removed on both sides after which it was allowed to regenerate without replacement from elsewhere. The operculum was formed on both sides but the gills failed to develop except in most rudimentary form—on the right side two rudimentary gills and on the left only a sharp conical process (Fig. 3). Blood circulating in these gills was first observed thirteen days after the operation. The larva was kept under observation for thirty-six days, at which age it was preserved. Its development, apart from the absence of gills, was normal.

The experiments are too few in number to warrant very definite conclusions. Absence of gills from both sides seems, in some cases at least, to lead to weakened heart action and ultimate stoppage of the circulation. On the other hand, the last case shows that it is possible for the embryo to adjust itself to the almost complete loss of its respiratory organs, sufficient aeration of the blood probably taking place through the general surface of the body.

Change of Orientation of the Branchial Ectoderm.

These experiments were for the purpose of determining whether there is a definite polarization of the ectodermal elements, and, further, whether any specific gill pattern is predetermined in the region. The experiments of the preceding sections already indicate that the latter is not the case.

Transplantations were made in four ways, as was done in the case of the experiments with limbs. The ectoderm was removed from the gill region and replaced by similar ectoderm either from the same or from the opposite side of the body and placed either with the dorsal or with the ventral border of the graft corresponding to the dorsal border of the wound. Besides these a few experiments were made in which the graft was rotated only 90°. The results may be stated very simply, for there is a marked tendency toward normal development in all combinations. Forty-eight experiments were made, twenty-eight of which gave definite results. Thirteen yielded normal gill-complexes, and these were distributed amongst all of the six different orientations tried. In five cases the second and third gills were normal but the first was small; in two cases the first gill was rudimentary while the others were normal; and in three cases the first gill was altogether lacking, the other two being normal. There were only three cases in which the gill-complex was irregular.

From these experiments the conclusion may be drawn that the branchial ectoderm is equipotential, and that there is no polarization of the elements affecting the potencies of differentiation. In this respect the branchial ectoderm of *Amblystoma* is markedly different from that of the anurans studied by Ekman.

In the latter changes in orientation of this layer are followed by corresponding changes in the position of the gills. This lack of axial differentiation in the gill ectoderm of the *Amblystoma* embryo lasts, however, for a brief period only, for in slightly later stages (from stage 29 on) change in orientation of the ectoderm is followed by marked disturbances in the arrangement of the gills.

Removal of the Mesoderm of the Branchial Region.

No satisfactory method of removing the mesoderm of the gill region and replacing it by other mesoderm has been worked out. However, it is a simple matter to remove all of this layer from the region in question and to cover the wound with clean ectoderm from the branchial region of another embryo. This affords the surrounding mesoderm an opportunity to wander in and fill the place taken by the original branchial mesoderm. The wound after such operations heals, though not nearly so readily as when there is mesoderm underlying the outer layer.

Sixteen such experiments were made, of which ten were successful. In only two of these cases was there any radical disturbance of the normal development of the gills. The other eight individuals developed approximately normally, though in five of them the heart was affected and the circulation was never established in the embryo. In three cases, however, the circulation was established on both sides, the gills of the operated side remaining smaller than the normal ones. In one the first gill was suppressed.

From the fact that in such a large proportion of cases the development of the circulation was radically interfered with, it seems likely that the removal of the mesoderm must have disturbed the material that goes to make up the heart. Aside from this the effect of the operation is slight. The mesoderm of the surrounding region obviously has the potency to fill in the gap made by the removal of tissue and then to take part in the development of normally formed, though at first smaller, gills.

Change of Orientation of the Branchial Ectoderm and Mesoderm.

These experiments are similar to those in which the ectoderm alone was turned, differing only in the circumstance that both ectoderm and mesoderm were lifted and implanted together. There is no difficulty in separating the mesoderm from the endoderm, though the healing of the wounds after transplantation is much less regular than when the ectoderm alone is taken. Inasmuch as turning the ectoderm alone has no effect upon the development of the gills, the effect of the transplantation of the two layers together must be due to the mesoderm alone. The respective results are strikingly different after the two operations.

There were sixty-three experiments in which both mesoderm and ectoderm were transplanted. Thirty-seven were positive. These cases were distributed unevenly amongst the different orientations, but the results throughout, except when the graft was from the same side of the body and normally oriented, were extremely variable and difficult to place in distinct categories.

The normally oriented grafts (homopleural dorsodorsal) gave five normal or nearly normal cases and one distinctly abnormal individual in which two gills were irregular and fused together.

In none of the other orientations were there any absolutely normal cases. However, in the inverted grafts from the opposite side of the body (heteropleural dorsoventral) there were three approximately normal individuals out of eleven positive experiments. Of the remaining eight cases, no two have been given the same designation except two which have been classified as very irregular and two others put down as rudimentary. Three gills were distinguishable in five of the eight cases, and only one gill was present in one. In one of the eight cases, and possibly in others, the abnormalities consisted in defects which might have been due to general conditions rather than to any particular disturbance of orientation.

Inverted grafts from the same side of the body (homopleural dorsoventral) gave not a single case, in a total of thirteen, that even approached the normal condition. Two gills were de-

veloped in five cases, but they were abnormal in some respects (Fig. 4); in the others only one gill was formed. Two individuals had quite irregular gills and three had rudimentary ones.

In the non-inverted grafts from the opposite side of the body (heteropleural dorsodorsal), of which there were only six available cases, the results were in no two cases exactly alike. In one a nearly normal complex developed. In two others three gills developed, but with abnormalities. The others were still less perfect.

The above results, standing alone, do not lead to any definite conclusion regarding the exact rôle of the mesoderm in the formation of the gills or regarding the nature of the disturbance caused by abnormal orientation of this layer. It is not clear that it is due simply to the reversal of a single polarized axis, such as is the case in the mesoderm of the anterior limb, although the fact that there is a certain tendency toward normal development in the inverted grafts from the opposite side of the body might be taken to indicate that the anteroposterior axis of the elements of this layer is more markedly polarized than is the dorsoventral. The experiments described in the next section point somewhat more clearly to such a conclusion.

Effect of Increasing the Amount of Mesoderm in the Branchial Region. Superposed Grafts.

In this group of experiments the branchial ectoderm was removed without materially injuring the underlying mesoderm, and a piece of tissue of the same size and shape including both mesoderm and ectoderm was healed over the wound. In this way the amount of mesoderm was approximately doubled, there having been only a very slight loss of mesoderm cells through the operation. The grafts were oriented in the four different ways as in the previous experiments.

The group with normal orientation (homopleural dorsodorsal) developed gills that were normal or nearly so in seven cases (Fig. 5), and in only one, in which the three gills were closely fused, was there any marked abnormality.

The inverted grafts from the opposite side of the body (hetero-

pleural dorsoventral) formed in all five cases gills which approached the normal condition. In two of these the gills were distinctly smaller than normal and in the other three there was considerable fusion and some irregularity, though all three gills could be distinguished.

On the other hand, the inverted grafts from the same side of the body (homopleural dorsoventral) showed in all five cases a very defective development of the gills, only one small, irregular or abortive gill being formed in each case.

In the non-inverted grafts from the opposite side of the body (heteropleural dorsodorsal) the results were likewise for the most part irregular or defective. Two fused gills developed in two cases, and in the other three only a single small, irregular or abortive gill was formed.

The results of these experiments show that while the doubling of the mesodermal material has in itself little or no morphogenetic effect, the orientation of the superadded material is of considerable consequence in determining whether aberrations in the development of the gills occur or not. Aside from experiments in which the transplanted material is normally oriented, these disturbances are apparently least marked in the case of inverted grafts from the opposite side of the body, and in this respect there is an approach to the condition found in the mesoderm of the fore limb bud. However, the results of this orientation of the grafted gill mesoderm are not normal with sufficient constancy to term the combination "harmonic", as was done in the case of the limb.

Removal of both Ectoderm and Mesoderm Without Replacement of Either.

This experiment is a by-product of the experiments described in the last two sections. The individuals are the donor embryos of those operations.

With such a large deep wound as is necessary in removing the gill mesoderm, a high mortality was to have been expected. Out of one hundred and six operations, only ten cases survived ten days or longer. Five others were preserved at intervals from an age of two to that of eight days.

The regeneration that takes place after this operation is very imperfect. In four cases a single fairly normal but small gill was regenerated, identified as the first gill in two cases, the second in one and the third in one. In three of these there were, in addition, one or two rudimentary gills. In the other cases either rudimentary gills alone were formed, or else no regeneration of gills took place at all. Blood circulation was established on the operated side in only two individuals. The circulation was defective throughout the embryo in four cases, as shown by the œdema that developed.

The imperfect restitution that takes place in these experiments must be ascribed to the severity of the operation rather than to the removal of anything specifically essential, for when either the mesoderm or the ectoderm alone is removed there is often complete restoration of function and structure.

Transplantation of Branchial Ectoderm.

The branchial ectoderm was transplanted either to the flank or to the anterior part of the head in a few cases, in order to test the potency of this tissue in strange surroundings. In all four individuals of the first group the results were essentially the same. Small nodules, which might possibly be interpreted as incipient gill sprouts, developed, but after a few days they became obliterated. In one case of the second group, where the gill ectoderm was transplanted to cover the eye and mandibular region, no supernumerary gills were formed and even the normal first gill failed to develop. On the other hand in two cases in which the graft was implanted not quite so far forward (Fig. 1, *A*) there were traces of rudimentary gills in the region of the hyoid and mandibular arches respectively. One of these (Fig. 6) showed two supernumerary filaments quite distinctly, in which, however, the circulation failed to become established. In two other cases, operated in a similar way, no supernumerary filaments were observed.

The potency of the gill ectoderm to form gills in any of these abnormal positions is obviously far less marked in *Amblystoma* than in the anuran species studied by Ekman, especially in *Rana fusca*.

Transplantation of Branchial Ectoderm and Mesoderm.

The results of this experiment are scarcely more definite than when the branchial ectoderm alone is transplanted. There is an initiation of development but it soon becomes arrested.

In five cases the whole of the branchial mound, including both ectoderm and mesoderm but no endoderm, was placed directly behind the normal gills, *i.e.*, in the region of or just ventral to the pronephros, the ectoderm and mesoderm having been previously removed. About the fourth or fifth day a marked prominence was present in the region of the graft, and, in two of the cases at least, distinct gill sprouts showed. There was, however, no further development; the grafted tissue gradually flattened out and became reduced to a slight hump or nodule.

In three other cases the graft was placed in front of the normal gills, in place of the material normally constituting the mandibular and hyoid arches. The results were not very different, except that the original gills of the embryo were to some extent disturbed in their development. No gills ever developed in front of the normal ones and in all three cases the balancer was suppressed.

Effect of Lack of Function on the Development of Gills.

It often occurs in experiments with amphibian embryos that the circulatory system does not function properly. Sometimes no movement of the blood can be observed even though the heart does beat. The effect of this upon the gills is noticeable; they never expand fully, and, while preserving their essential character, they have an atrophic appearance.

In experiments on the gills in particular it not infrequently happens that, although the embryo is otherwise normal, the circulation in the operated gills is either delayed or fails altogether to become established. The same atrophic appearance is found in such cases.

When the heart is removed a similar condition ensues as regards the gills, but the effects on the embryo as a whole are much more pronounced. It becomes badly swollen and ulti-

mately dies. Operations of this kind were done upon embryos in stages 33 and 34, *i.e.*, just before the heart begins to beat. It is difficult to remove the heart rudiment completely. There is almost always some regeneration, and pulsation is established, which, however, is usually ineffective and in such cases no blood can be seen in movement in the yolk vessels or through the gills. Nevertheless, the latter grow out, and are normally constituted except for their atrophic appearance. The operculum and the gill plates are also formed. As regards the development of their specific form, the gills, like many other structures, are thus independent of function, though the marks of atrophy in the functionless organs are unmistakable.

Conclusion and Summary.

So far as the present experimental analysis has led, the respective rôles of ectoderm and mesoderm in the formation of the gills are as follows:

The branchial ectoderm is specifically gill forming, but surrounding the gill region, and, more particularly, posterior to it, the ectoderm has the potency to form gills in a diminishing intensity as the distance from the gills increases. This is shown both by transplantation experiments and by regeneration after removal of the branchial ectoderm.

The gill pattern is not, however, laid down in the branchial ectoderm, as is shown by the fact that this same ectoderm, when turned in any way or even when taken from the opposite side, gives rise to normal gills notwithstanding.

The specific pattern must therefore be determined by the deeper layers, but in the absence of experiments upon the endoderm, the exact rôles of mesoderm and endoderm, respectively, cannot be determined. The indications are, however, that the endoderm does not play merely a passive part, as Ekman maintains to be the case in the anurans.

The mesoderm cannot be a well-defined mosaic, for doubling of the material by superposition, provided the orientation of the grafted tissue is normal, does not disturb the normal development of the gills. Abnormal orientation of the mesoderm, however,

both after extirpation of the normal mesoderm and in cases of superposition, results in marked aberration from the normal development, showing that this layer is not without axial differentiation. These aberrations from the normal are not specific for any particular orientation, but there is a greater degree of disturbance after the orientations which, in the case of the fore limb, have been found to be disharmonic, *i.e.*, inverted mesoderm from the same side of the head and non-inverted from the opposite side. One of the combinations called harmonic in the case of the fore limb (that in which the graft is normally oriented) gives normal gills, but the other, while yielding a considerable number of cases of approximately normal gills, results on the other hand in a good many irregularities. These circumstances may be taken to indicate that the gill-mesoderm elements are distinctly polarized in an anteroposterior direction and perhaps less markedly so dorsoventrally. The fact that the mesoderm from the periphery of the gill region is capable of forming gills, taken together with the fact that doubling the amount of the material does not disturb normal development, points to the conclusion that this mesoderm is equipotent.

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Explanation of Plate I.

Figure 1. Profile view of an embryo of *Amblystoma punctatum*, stage 21. The various flaps of ectoderm used in the transplantations are indicated by ovals. A, includes the mandibular, hyoid and anterior part of the branchial region; BR, branchial region; C, ventroanterior part of head region; C′, head region; CD, cardiac region; Fl, flank; PN, pronephric and limb region. × 10.

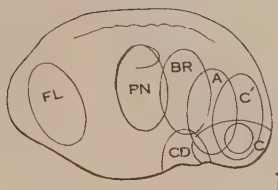
Figure 2. Replacement of branchial ectoderm by ectoderm from the flank (Exp. E. Br. E. 21) resulting in complete suppression of gills on the right side; ventral view of larva eighteen days after operation. × 10.

Figure 3. Larva with gills almost suppressed through failure to regenerate after removal of branchial ectoderm (Exp. E. Br. M 15–); specimen preserved thirty-six days after operation. × 10.

Figure 4. Larva with abnormal gills on the right side due to inversion of the branchial ectoderm and mesoderm (Exp. Br 35), specimen preserved fifteen days after operation. × 10.

Figure 5. Individual showing normal gills developed from double quantity of mesoderm (Exp. S. Br. 17); specimen preserved ten days after operation. × 10.

Figure 6. Individual in which branchial ectoderm (Fig. 1, BR) had been transplanted to a position just anterior to gill region (Fig. 1, A) (Exp. Br. A 2); two supernumerary gills (H and M) developed; the dotted line indicates the boundary of the transplanted ectoderm; sketch made ten days after operation. × 10.



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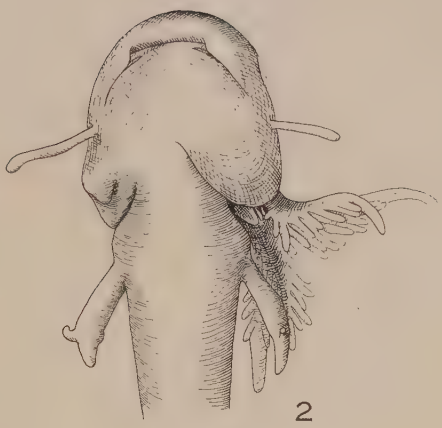

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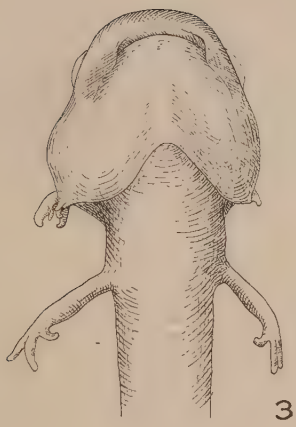

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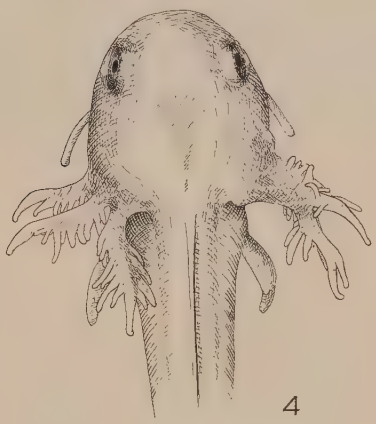

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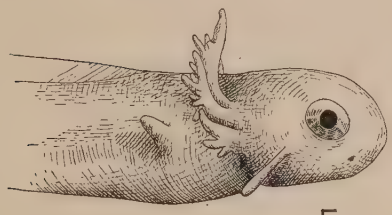

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ROSS G. HARRISON.

[Reprinted from The American Naturalist, Vol. LV., September–October, 1921]

STANDARDIZED MICROPHOTOGRAPHY

Third Contribution: The Exposure Factor

The great convenience to be derived from the use of only two tables of factors is so apparent that anybody who is at all familiar with microphotographic work should not for a moment hesitate to spend some time in the preparation of such tables. It is, of course, natural that differences in the source of light, optical equipment and ray-filters necessitate the preparation of new tables. Our own outfit being one of the standard Bausch and Lomb apparatus with a Zeiss microscope and Cramer ray-filters, it occurred to me that those possessing a similar equipment might be spared the tediousness of preparing an exposure table if our table of exposure factors were published with such instructions as would permit of identical arrangement of the apparatus. Moreover, it will be remembered that these factors are dependent upon the source of light, substage position, magnification, and numerical aperture of the objective, and that other conditions being identical, exposure varies as the square of the numerical aperture.

A word must be said about the use of the substage condenser. The position of the condenser indicated in the table is such as to give the greatest detail without *apparent* bad effect on definition. As is well established, the depth of focus in a microscopic objective depends upon its numerical aperture. The greater the latter, the smaller the depth of focus. Since definition increases with numerical aperture, one has to sacrifice the one or the other. From a practical point of view depth of focus is often more desirable than perfect definition. The numerical aperture of an objective may be conveniently cut down by increasing the distance between the substage condenser and the objective. This can be done with safety only to a certain point, which I called the optimum, and beyond which definition is visibly impaired. I hope to be able later to return to this subject in greater detail. Meanwhile, we may state as a general rule that the higher the magnifying power of an objective, the sooner the optimum will be reached. With other words, the substage may be lowered a great deal more in low-power than in high-power objectives.

In the following table the position of the substage is indicated in millimeters, assuming that it is at zero when moved up as far as it will go. In the Zeiss microphotographic stand the substage condenser has a numerical aperture of 1.40, and when placed at zero the surface of its upper lens is still 0.8 mm. below the surface of the microscopic table.

The source of light for which the table of exposure factors holds good is the Bausch and Lomb Microprojector No. 4301 with hand feed arc lamp and rheostat for 4.5 amperes, 110 volts, D.C., placed so that the distance between the uprights carrying the projector and the microscope table is 20 inches, while halfway between the projector and the microscope a water cell is placed for the absorption of heat rays. The projector has a movable condenser of its own. The position of this condenser influences the intensity of light. Our table is made for such a position of this condenser that the light on the focusing screen is brightest. It is not the same for every objective and must be found by experimenting, with the aid of an assistant, who moves the condenser while the observer watches the field. For this experiment the microscope substage is best lowered about 2 mm. to insure even illumination. When the position of the condenser of the projector has been found and marked on the mounting, the experiment need never be repeated again. The table itself applies only to an Orthonon plate used without ray-filter. For use with Cramer's rayfilters the factors given in this table must be multiplied by the factors shown in my first paper.

The so-called "Pointolite" is an excellent source of light for low powers. It requires about 5–10 times longer exposures. In our laboratory we use the Complete Illuminating Apparatus with D.C. open arc. After some experimenting it was found that the most satisfactory arrangement of this illuminating system is when the arc is moved forward to within 2½ inches from the first condensing lens, a water cell placed between second and third condensing lenses, in contact with the mounting of both, and the distance between the third and fourth condensing lens fixed at 15 inches, while the distance from the latter to the microscope table is 7½ inches. Under these conditions the exposure is double that obtained with the microprojector for which the table in this paper is given. I omit giving a separate table for this system, because any one may obtain it in a moment by simply doubling the values given here. But it must be re-

TABLE OF EXPOSURE FACTORS FOR ORTHONON PLATES WITHOUT RAY-FILTER, USED WITH A B. & L. OPEN ARC D.C. 110 VOLT MICROPROJECTOR AND AN ABBE SUBSTAGE CONDENSER WITH N.A. 1.40.

Linear Magnification.	B. & L. Achromat E. F. 32 N. A. 0.10 Substage completely removed.	B. & L. Achromat F. F. 16 N. A. 0.25 Substage lowered 8 mm.	Zeiss Apochromat F.F.16 N. A. 0.30 Substage lowered 8 mm.	B. & L. Achromat E. F. 8 N. A. 0.50 Substage lowered 5 mm.	Zeiss Apochromat E. F. 8 N. A. 0.65 Substage lowered 5 mm.	B. & L. Achromat F. F. 4 N. A. 0.85 Substage lowered 4 mm.	Zeiss Apochromat E. F. 4 N. A. 0.95 Substage lowered 4 mm.	B. & L. Achromat E. F. 1.9 N. A. 1.30 oil immersion Substage lowered 1.5 mm.	Zeiss Apochrom't E. F. 2 N. A. 1.30 oil immersion Substage lowered 1.5 mm.
25	0.0005								
30	0.0007								
40	0.0013								
50	0.0020								
75	0.0045	0.013	0.011						
100	0.0080	0.024	0.020						
125		0.037	0.031						
150		0.054	0.045						
175		0.073	0.061						
200		0.096	0.080						
250		0.150	0.125						
300		0.216	0.180	0.108	0.090	0.048	0.040		
350		0.294	0.245	0.147	0.122	0.065	0.054		
400		0.384	0.320	0.192	0.160	0.085	0.071		
500				0.300	0.250	0.133	0.111	0.040	
600				0.432	0.360	0.192	0.160	0.058	
700				0.588	0.490	0.262	0.218	0.078	
800						0.351	0.284	0.102	
900						0.432	0.360	0.130	
1000						0.532	0.444	0.160	
1250								0.250	
1500								0.360	
1750								0.490	
2000								0.640	
2500								1.000	
3000								1.440	

membered that change in the arrangement of the illuminating system, no matter how small the deviation, will result in wrong values.

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[Reprinted from BIOLOGICAL BULLETIN, Vol. XLI., No. 3, September, 1921]

THE STRUCTURE, LIFE HISTORY, AND INTRAGENERIC RELATIONSHIPS OF PARAMECIUM CALKINSI, SP. NOV.

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(SEVEN FIGURES.)

The term *Paramecium* was coined by Dr. John Hill of London in 1752 for a group of animalcules comprising "four species," two of which probably include members of the genus *Paramecium* as recognized today.[1] Then O. F. Müller in 1773[2] named an organism *Paramœcium aurelia*, and in 1786,[3] in the first comprehensive attempt to classify scientifically microscopic animals, definitely established the genus *Paramecium* and included under it five species. Unfortunately, however, without the slightest warrant etymological or otherwise, Müller changed the spelling of *Paramecium* to *Paramœcium*. Among the species which Müller distinguished are *Paramœcium aurelia* and *Paramœcium versutum*. The former apparently is Hill's "species 3" and either the present-day *P. aurelia* or *P. caudatum*. *Paramœcium versutum* possibly is Hill's "species 1" and apparently the present species *P. bursaria*. Müller's other paramecia have since been relegated to other genera.

Ehrenberg, in his great monograph on the Infusoria[4] (1838), recognized eight species under the genus and returned to the spelling of Hill with the note "Paramecium ist richtiger als Paramœcium." Among Ehrenberg's species are two true paramecia: *Paramecium aurelia*, which he assumed to be the *P. aurelia* of Müller, and *P. caudatum*, which he distinguished from *P. aurelia* chiefly by the greater attenuation of the posterior end of the animal. Müller's species *P. versutum*, to which Focke in the meantime[5] had given the name *Paramecium bursaria*, Ehrenberg placed under the genus *Loxodes* as *L. bursaria*.

[1] "History of Animals." London, 1752.

[2] "Vermium terrestrium et fluviatilium Historia." Havniae et Lipsiae, 1773.

[3] "Animalcula Infusoria fluviatilia et mariná." Havniae, 1786.

[4] "Die Infusionsthierchen als volkommene Organismen." Leipzig, 1838.

[5] "Ueber einige Organisationsverhältnisse bei polygastrischen Infusorien und Rädertieren." *Isis*, 1836.

In 1841 Dujardin[6] recognized only two species of *Paramecium*, *P. aurelia* and *P. caudatum*, and placed *P. versutum* of Müller (= *P. busaria* of Focke = *Loxodes bursaria* of Ehrenberg) in the genus *Bursaria*, though apparently with some hesitancy.

Claparède and Lachmann in their series of studies (1858–1860)[7] distinguished eight species of *Paramecium*, including *P. putrinum*, a new species which is still recognized, *P. bursaria* and *P. aurelia*. They abolished *P. caudatum* with the remark "Le *P. caudatum* Ehr. n'en est certainement pas spécifiquement différent. C'est une simple variété qu'on pourrait même à bon droit considérer comme la forme typique de l'espèce." [8]

Thenceforth, for about thirty years, the name *Paramecium aurelia* was applied indiscriminately to both *P. aurelia* and *P. caudatum*. Most of the pioneer studies on conjugation, etc., were made, as we know now, on *P. caudatum*, though it was called *P. aurelia*. Then came the first hint which was to straighten out the matter. Maupas in 1883[9] noted a difference in the micronuclear apparatus of certain paramecia which led him in 1888[10] to announce definitely that the structure and number of the micronuclei is a distinctive character and thus on this basis to reëstablish the two common species of *Paramecium*. Since 1889, when both Maupas[11] and Hertwig[12] studied the nuclear phenomena during conjugation of these forms, *P. aurelia* and *P. caudatum* generally have been regarded as distinct species, and the validity of this conclusion has been emphasized by Jennings and Hargitt,[13] and by Woodruff[14] using pedigree culture methods.

Studies on the conjugation phenomena of *P. bursaria* by

[6] "Histoire naturelle des Zoophytes, Infusoires, etc." Paris, 1841.

[7] "Études sur les Infusoires et les Rhizopodes," Geneva, 1858–1860.

[8] *Ibidem*, part I., p. 265.

[9] "Contributions a l'etude morphologique et anatomique des Infusoires cilies," *Arch. de Zool. Exp. et Gen.*, (2), I., 1883, p. 660.

[10] "Sur la multiplication des Infusoires cilies," *Arch. de Zool. Exp. et Gen.*, (2), 4, 1888, p. 231.

[11] "Le rajeunissement karyogamique chez les cilies," *Arch. de Zool. Exp. et Gen.*, (2), 7, 1889.

[12] "Ueber die Konjugation der Infusorien," *Abh. Kgl. bayr. Akad. d. Wiss.*, München, 2, Cl. 17, 1889.

[13] "Characteristics of the diverse races of *Paramecium*," *Journ. Morph.*, 21, 1910

[14] "*Paramecium aurelia* and *Paramecium caudatum*," *Journ. Morph.*, 22, 1911.

Hamburger[15] and of *P. putrinum* by Doflein[16] apparently leave no question in regard to the validity of these species, though both species are remarkably similar.

In addition to *P. aurelia*, *P. caudatum*, *P. bursaria* and *P. putrinum*, which are accepted by Schewiakoff in his monographic survey of the holotrichous Infusoria,[17] there must be mentioned *Paramecium trichium* described by Stokes[18] in 1885, and *P. multimicronucleata* by Powers and Mitchell[19] in 1910.

Stokes discovered *P. trichium* in the zoöglœa on an infusion of dead leaves. His description, in part, is as follows:

"It seems to be a distinct species, and one that can scarcely be mistaken for any known form, except possibly for *P. bursaria* (Ehr.) S. K., differing from the latter, however, conspicuously in form, especially in the apparently oblique curvature of the anterior extremity, in the absence of the truncation of the same part, the absence of the rapid and continuous circulation of the endoplasmic contents, and particularly the green coloration of the cortex and sarcode. The two contractile vesicles, instead of being placed one in each body-half, as in *P. bursaria*, are here anterior and close together, contracting quickly, the one beginning to again form almost before the completion of the other's systole. Trichocysts are very abundant, and are so arranged that they seem to elevate the cuticular surface into the minute hemispherical bosses that cover the entire body. When forcibly extruded through the influence of the glycerole of tannin, the distal end of each, for about one tenth of the entire length, is conspicuously thickened, so that the trichocyst seems to be supplemented by the addition of a minute pyramid."

Schewiakoff considers *P. trichium* to be identical with *P. putrinum*, but if Stokes' description is accurate there is no warrant for this conclusion. Certainly further data are needed before a final answer can be given.

[15] "Die Konjugation von *Paramecium bursaria* Focke," *Arch. f. Protistenk.*, 4, 1904.

[16] "Lehrbuch der Protozoenkunde," 2 Auflage, Jena, 1909.

[17] "Organization et classification des infusoires *Aspirotricha* (*Holotricha auctorum*)," *Mem. Acad. Imp. Sci.*, St. Pétersbourg, Classe d. Sci. Phys. et Math., (8), 4, 1896.

[18] "Some New Infusoria," *Amer. Naturalist*, XIX., 1885.

[19] "A New Species of *Paramecium* (*P. multimicronucleata*) Experimentally Determined," BIOL. BULL., 19, 1910.

P. multimicronucleata, as the name indicates, characteristically has many small micronuclei, usually from six to nine, the structure of which clearly resembles that of the micronuclei of *P. aurelia*. The authors' figures show a general cell-form which is more or less intermediate between that which is typical for *P. aurelia* and for *P. caudatum*. Powers and Mitchell emphasize the persistence of the specific characters after conjugation. It is an interesting form which warrants further study, especially in view of the fact that we now know that reduplication of micronuclei occurs during endomixis.

In January, 1919, I isolated a ciliate, from an infusion of fresh water and vegetable débris collected at New Haven, which, though undoubtedly a *Paramecium*, showed characters that distinguished it from any of the species hitherto described. It has now been extensively studied in pedigree cultures for more than a year and it has bred true. I have, therefore, definitely designated it a new species, and named it *Paramecium calkinsi*[20] in recognition of the fact that Professor G. N. Calkins of Columbia University introduced students of the Infusoria to exact daily isolation pedigree culture methods. *Paramecium calkinsi* presents an interesting composite picture of characters of *P. aurelia*, *P. bursaria*, *P. putrinum* and *P. trichium*, with certain distinctive ones of its own.

The general body form of *Paramecium calkinsi* is more similar to that of *P. bursaria*, *P. putrinum* and *P. trichium* than to that of *P. aurelia* and *P. caudatum*. Indeed, at first glance, one is reminded of *P. bursaria*, and further study suggests *P. putrinum* and *P. trichium* because individuals in different stages of nutrition and dividing at various rates exhibit quite a different appearance. The form which is most characteristic—in fact diagnostic—when the living cells are studied day after day in pedigree cultures is given somewhat diagrammatically in Figs. 1 to 4.[21]

The cells of this pedigree culture of *P. calkinsi*, under favorable food conditions, average about 120 μ in length and 50 μ in

[20] L. L. Woodruff, "*Paramecium calkinsi*, sp. n.," *Proc. Soc. Exp. Biol. & Med.*, Feb., 1921.

[21] Drawings made by Miss J. E. Lovett of this Laboratory.

breadth. In other words, the length is about the same as that of many races of *P. aurelia*, but the breadth is proportionally

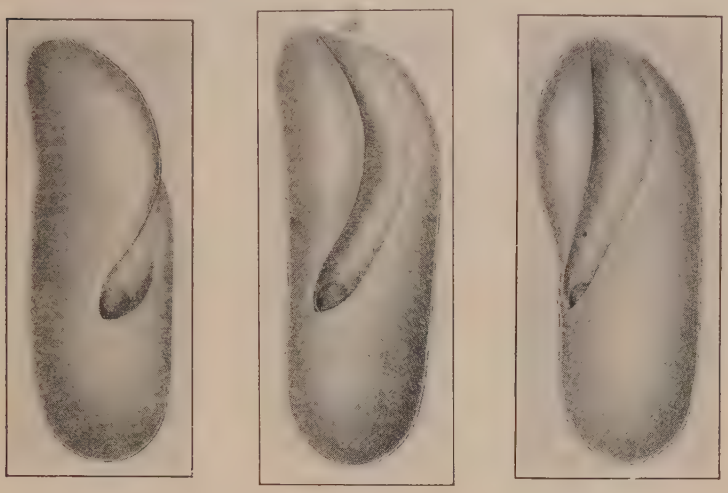

FIG. 1. FIG. 2. FIG. 3.

FIGS. 1–3. *Paramecium calkinsi*, sp. nov.

greater and the broadest part is anterior, whereas it is posterior to the middle in *P. aurelia*. But, of course, no emphasis can be placed on size relations since it is probable that when *P. calkinsi* from other localities is studied, racial size differences will be found to occur.

The ciliary apparatus of the new species affords no striking characteristic and an undulating membrane is present in the gullet as in all paramecia. The trichocyst apparatus is particularly well developed. The trichocysts are relatively evenly distributed in the cortical layer of protoplasm, except at the anterior end where they are more closely crowded together. The surface of the cell in profile presents, upon careful study, a slightly crenulated outline, each of the tiny elevations representing the position of a trichocyst and the depressions between affording the points of origin of the cilia. The structure of the trichocysts as they appear in situ is shown in Fig. 4. Exploded and detached examples appear in Fig. 5.

It will be noted that the trichocyst apparatus of *P. trichium*, as decribed by Stokes, and which he emphasized in naming the organism, agrees closely with that in the new species. The one essential difference is the form of the exploded trichocysts, which in *P. calkinsi* have almost the identical structure of those in *P. caudatum* as described by Khainsky.[22]

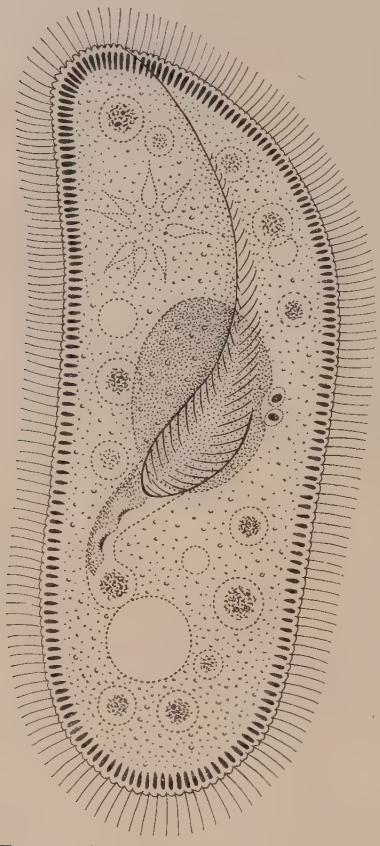

FIG. 4. *Paramecium calkinsi*, sp. nov.

The endoplasm of the new species is characteristically more vacuolated than that of *P. aurelia* or *P. caudatum* when all are bred in the same culture medium. On the other hand it is by no means so fluid as in *P. bursaria*. It is possible to distinguish almost at a glance the new species from the others, merely by the cytoplasmic appearance, when all are mingled together in the same culture.

The contractile vacuoles, as the figure shows, are two in number, and agree closely in position and form with those typical of *P. aurelia*, *P. caudatum*, and *P. bursaria*. Herein is a striking difference from *P. putrinum* with one contractile vacuole at the anterior end and *P. trichium* in which two contractile vacuoles are situated close together near the anterior end.

The macronucleus presents no diagnostic characteristics, though, as the figure shows, in the culture under consideration it usually is proportionately larger in size than it is in the well-known species of *Paramecium*. Little emphasis, however, can

[22] A. Khainsky, "Zur Morphologie und Physiologie einiger Infusorien," *Arch. f. Protistenk.*, 21, 1910.

be placed on this as the size relations vary in all races of all species under different conditions. But I have never seen the macronucleus of *P. calkinsi* as small as Stokes figured it for *P. trichium*.

The micronuclear apparatus is almost identical with that of *P. aurelia* and in marked contrast with that of the other species (see Figs. 4 and 6). In the first place, there are characteristically

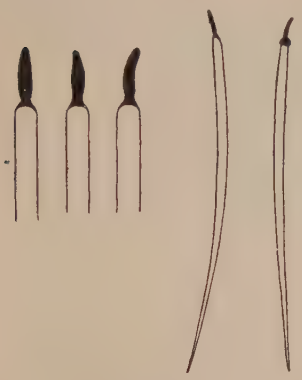

FIG. 5.

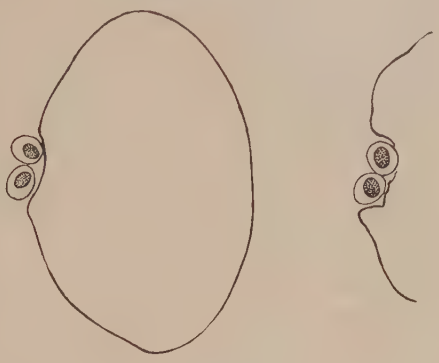

FIG. 6.

FIG. 5. 'Exploded' trichocysts of *Paramecium calkinsi*.

FIG. 6. Micronuclear apparatus of *Paramecium calkinsi*. Left, from a total preparation; right, from a section. Camera lucida sketches.

two micronuclei situated in or near a slight depression of the macronucleus. It has not been possible in every case to discover two micronuclei but there can be no doubt that two are typical of the vegetative cells. And, of course, as in all paramecia, the position of the micronuclei varies considerably in different individuals. Sometimes the macronuclear depression is on one side and sometimes on the other side. Frequently there are two tiny depressions each with a micronucleus. Again, the micronuclei may be at a considerable distance from the macronucleus, though not in mitosis.

In the second place, the relative size and the structure of the micronuclei are essentially similar to those of *P. aurelia*. The chromatin of the resting micronuclei is clumped to form an endosome which is surrounded by an 'achromatic' layer enclosed by a distinct nuclear membrane.

The general behavior as well as the 'avoiding reaction,' etc., of *P. calkinsi* is very similar to *P. caudatum* and *P. aurelia*, though the broad and slightly curved anterior end of *P. calkinsi* renders the revolutions on its long axis and its spiral path somewhat more conspicuous than in the more common species of *Paramecium*.

Paramecium calkinsi appears to be adapted to essentially the same environmental conditions as *P. aurelia* and *P. caudatum*. A series of experiments in which flasks of hay infusion were seeded with both *P. calkinsi* and *P. aurelia* showed that, as a rule, one and not both species attained a maximum development in the same flask. Usually within a few days there was a heavy growth of one and only a few struggling survivors, or none at all, of the other species. There was no indication that one was more hardy than the other under the conditions of the experiment; success or failure to dominate the culture apparently depended on which form 'got the start,' so to speak. It seems probable that the excretion products of the two forms are mutually detrimental and that this is the determining factor in the comparative incompatibility of the two species.[23] This point is being investigated.

The rate of reproduction of the new species, as exhibited in pedigree cultures throughout nearly a year, shows no factors which distinguish it from *P. aurelia*, *P. caudatum* or *P. bursaria*, which I have studied under similar conditions, since it is well known that characteristic division rates obtain in different races of the same species. The main pedigree culture of *P. calkinsi* was carried from January 30 to November 20, 1920, when it died out at the 321st generation (Fig. 7). During all this period a constant culture medium of standard beef extract was employed, and during certain experiments the temperature was maintained, by a thermostat, practically constant at 24° C. The race is now thriving in mass cultures which were seeded from the pedigree lines. The animals are apparently in as healthy condition as ever.

[23] L. L. Woodruff, "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions," *Jour. Exper. Zoöl.*, 14, 1913.

Throughout the year's work every effort has been made to secure conjugation and encystment in the mass cultures, though without result. Furthermore, an intensive study, involving the making of more than a thousand permanent preparations of pedigree animals at all stages in the life of the culture,[24] has failed to reveal any indications of endomixis. Therefore, it can be stated positively that endomixis has not occurred in this culture, although there are fluctuations of the division rate somewhat similar to those associated with endomixis in *P. aurelia* and *P. caudatum*.[25] This point will be considered in detail in another paper.

As is well known, the diet of *Didinium nasutum* is almost exclusively confined to paramecia, and it has not been possible to cultivate successfully the former without this food. An experiment extending through several months showed that *Didinium* thrives on *P. calkinsi* just as well as on *P. aurelia*—further evidence that the new species is a *Paramecium!*

From the foregoing description of the structure and life history of *P. calkinsi* it is apparent that this

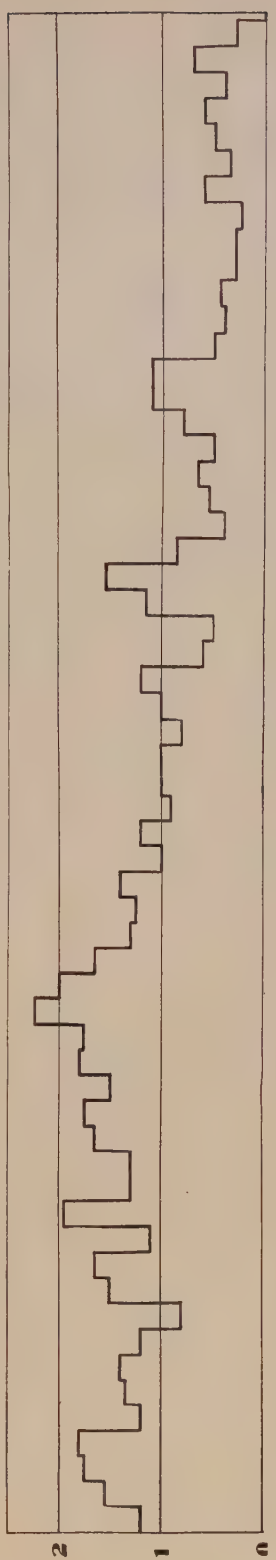

FIG. 7. Graph of the division rate of the main pedigree culture of *Paramecium calkinsi* from January 30 to November 20, 1920. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for five-day periods.

[24] I am indebted to Miss Hope Spencer of the Osborn Laboratory for assistance in carrying out this work.

[25] Woodruff and Baitsell, "Rhythms in the Reproductive Activity of Infusoria," *Jour. Exper. Zoöl.*, 11, 1911. Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exper. Zoöl.*, 17, 1914. Erdmann and Woodruff, "The Periodic Reorganization Process in *Paramecium caudatum*," *Jour. Exper. Zoöl.*, 21, 1916.

species presents a complex mosaic of the characters of the other members of the genus[26]—indeed the intrageneric relationships of the new species are its chief interest.

A survey of the six species of *Paramecium* under discussion shows that they fall into two quite clearly defined groups which may be referred to as the '*aurelia* group' and the '*bursaria* group' respectively. The members of the '*aurelia* group' (*P. aurelia*, *P. caudatum* and *P. multimicronucleata*) are characterized by a relatively long spindle- or cigar-shaped body; those of the '*bursaria* group' (*P. bursaria*, *P. putrinum*, *P. trichium* and *P. calkinsi*) by a somewhat shorter and broader form, with a tendency, especially prominent in *P. bursaria*, toward a dorsoventral flattening.

Within each of these groups are found two general structural types of micronuclei. One type, which may be termed the '*caudatum* type,' is exhibited by *P. caudatum* in the '*aurelia* group,' and by *P. bursaria*, *P. putrinum* and apparently by *P. trichium* in the '*bursaria* group.' Though Stokes' figure of the micronucleus of *P. trichium* leaves much to be desired, it clearly indicates that the single micronucleus is not of the 'endosome' type. The other type of micronucleus, the 'endosome' or '*aurelia* type,' occurs in *P. aurelia* and *P. multimicronucleata* in the '*aurelia* group,' and in *P. calkinsi* in the '*bursaria* group.' All species with the '*caudatum* type' characteristically possess one micronucleus, and those with the '*aurelia* type,' two micronuclei, except *P. multimicronucleata* in which the number apparently varies from six to nine.

Paramecium calkinsi represents the '*aurelia* type' of micronuclear apparatus in the '*bursaria* group' of species. It is possible that future study may show that certain of the paramecia under discussion (*e.g.*, *P. micronucleata*, *P. trichium*, *P. putrinum*) should be regarded as racial forms rather than distinct species, but I believe that the data presented above indicate beyond doubt that *Paramecium calkinsi* takes its place by *P. bursaria*, as *Paramecium aurelia* has by *P. caudatum*, as a distinct species.

[26] It is unnecessary to illustrate the cell form and micronuclear structure of the several species of *Paramecium* because figures are available in many of the monographs cited. Especial reference should be made to the figures given by Jennings and Hargitt, Schewiakoff, and Hamburger.

March 4, 1921.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 26

Reprinted from THE ANATOMICAL RECORD
Vol. 22, No. 3, October, 1921

SITUS INVERSUS VISCERUM IN DOUBLE TROUT

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SIX FIGURES

This investigation on situs viscerum was undertaken at the suggestion of Professor Harrison, to whom the writer gladly acknowledges his indebtedness for helpful criticism and advice. The material consists of a small collection of trout embryos (Salmo fario) taken by Prof. A. Petrunkevitch in Freiburg in 1901, a number of which show abnormalities of different kinds. The results are presented in the hope that they may prove of value in supplementing previous observations, particularly those of Morrill ('19), who made use of similar material. For this reason, then, very little attempt has been made to explain the extraordinary conditions of the viscera; they are merely described briefly and the relationship of the degree of external and internal doubling to the occurrence of situs inversus, in so far as it is disclosed by these animals, is shown.

From these trout embryos, fifteen which showed doubling to a greater or less degree were isolated. The specimens had been fixed in corrosive sublimate and preserved in 70 per cent alcohol, and it was found necessary to run them back to water before the yolk mass, which in each case united the two components, could be dissected off without damage to the underlying tissues. The region of fusion of the vertebral columns is taken as the criterion of the amount of external doubling, that of the intestinal tracts of internal doubling. The embryos show a fairly complete gradation in amount of doubling from almost total separation of the vertebral columns to a very close union as parasite and autosite. These terms are used to denote, respectively, the smaller and larger of the two unequal compo-

nents of the same monster. In every case one abdominal cavity is common to the two components, the ventral body walls being fused around a common yolk mass. In some cases there is such distortion of the viscera that it is almost impossible to decide whether the situs is normal or not. The asymmetry of the hearts could not be made out with certainty. The dissections were all made under the low power of the binocular and the drawings are from camera-lucida sketches of these dissections (magnification, × 8.) The nomenclature adopted by Morrill ('19) for the components of the monsters is followed in each case i.e., designating as A the right twin (at the observer's left in a ventral view), and as B the left twin (right from the ventral aspect). The components are thus referred to in the descriptions, table, and figures.

MORPHOLOGICAL FINDINGS

The embryos are arranged in this section in the order of their degree of external doubling.

No. 1. The components of this monster are united by their ventral surfaces so that their median planes are almost coincident. Component A is slightly larger than B, showing a higher degree of development. The right eye of A is normal, the left has a rudimentary lens, as does the right eye of B, while the left eye of B is not visible externally. The vertebral columns of both components are separate to near the base of the tail, where the body musculature fuses more completely, forming a single tail. There are, however, two dorsal and two caudal fins. The abdominal cavity contains the viscera of the two components situated on diametrically opposite sides of the yolk mass. The intestinal tracts are separate until just before they open at the anus; during the passage from the abdominal cavity to this point they are closely associated side by side as parallel tubes.

The situs of the gut of A is normal, the stomach curving first slightly to the left, then making a wider curve to the right to pass over into the intestine which goes straight caudad to the anus. The liver lies on the right side of the intestinal canal.

The swim bladder, after its origin from the dorsum of the gut well toward the anterior end of the coelom, lies on the left of the canal. The curvatures of the gut of B are normal, as is also the liver, though this organ extends ventrally across the gut farther than normal, displacement being due probably to the pressure of the yolk mass. It sends a small pointed lobule cephalad on the right side of the stomach. The swim bladder of B lies on the right side of the gut immediately after its origin from the dorsal surface and continues in this position to its termination beneath the liver. This abnormal position may also have been due to the pressure of the yolk mass.

No. 2. The vertebral columns are separate to nearly the tip of the tail, the caudal fin is single. The head of A is slightly larger than that of B. The gut of each is normal, showing normal curvatures, and is single to a point slightly anterior to its exit from the body cavity. Here the intestines of the two components fuse. The liver of B is normal in size, shape, and position. That of A is rotated to a more transverse position than usual, otherwise it is normal. The swim bladders are both normal in position.

No. 3 (fig. 1). This specimen shows a typical case of situs inversus in the right twin (component A). The vertebral columns are fused posterior to the pelvic fins, the components are of nearly equal size, externally normal, and so placed that the dorsal fins lie almost in the same plane. The gut passes down the left and right sides of the body walls of components A and B, respectively, to a point about halfway between the pylorus and the exit from the body cavity, where the two fuse into one. Then the common intestine crosses to the ventral side of the coelom and passes back to the anus, where it opens to the outside. The shape of the two stomachs, livers, and swim bladders is normal, but these organs in component A are mirror images of those in component B. The liver of B lies on the right side of the stomach and ventral to the first part of the intestine, with its convex border toward the right; the greater curvature of the stomach is towards the left and is paralleled by the swim bladder. The stomach of A, on the other hand, has its greater

curvature toward the right, and the swim bladder is also on this side. The liver, with its convexity toward the left almost in contact with the corresponding part of the liver of B, is on the left side, its posterior end swinging to the right across the pylorus and the beginning of the intestine.

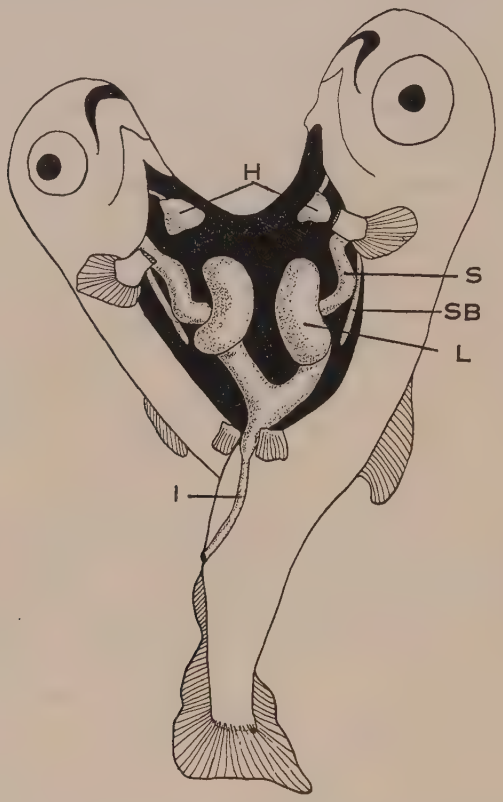
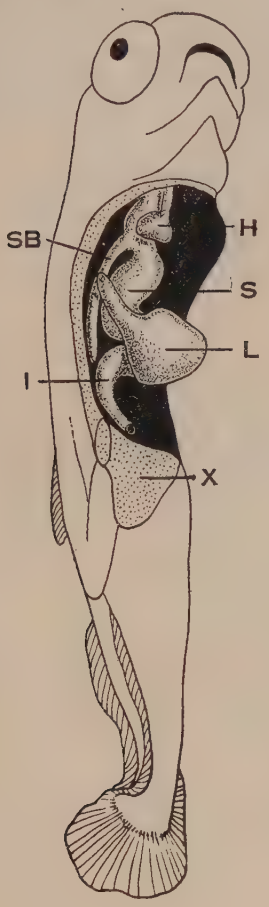


Fig. 1 Ventrolateral view, specimen no. 3. The situs viscerum B is normal, A is reversed. *H*, heart; *L*, liver; *SB*, swim bladder; *S*, stomach; *I*, intestine.

No. 4 (fig. 2 and 3). Situs inversus viscerum of the left twin is clearly shown in this embryo. The two components are of equal size and are placed in almost exact ventral apposition. It was found necessary to cut them apart to show the internal anatomy. The vertebral column of each is single to a point

slightly posterior to the dorsal fin, where the myomeres of the contiguous sides become fused. The adipose fins are separate, though one caudal fin is common to the two components.

The gut of component A is obviously in normal situs, though there is a considerable curvature of the cardiac end of the stomach



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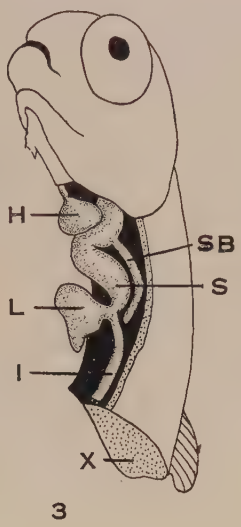

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Fig. 2 Component B of specimen no. 4, ventrolateral view. *X* marks the point of separation from component A (fig. 3), other abbreviations as in fig. 1. This twin shows situs inversus.

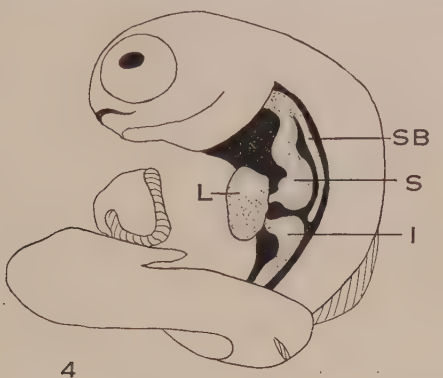
Fig. 3 Component A of specimen no. 4, ventrolateral view. Situs is normal. Abbreviations as in figs. 1 and 2.

ventrad and to the right caused by the pressure of the yolk mass upon it. The gut is single until just before its exit from the abdominal cavity, at which point the intestines of the two components fuse and pass to the anus as a single tube. The liver of component A, smaller than normal and somewhat wedge-shaped, occupies a ventral position a little posterior to and to the right of the middle of the common abdominal cavity. It is placed transversely with reference to the gut on the right side of the pylorus. The swim bladder of A is in its normal position at the left of and parallel to the stomach.

The stomach of component B, on entering the abdominal cavity, passes diagonally to the left for a short distance, then, just beyond the origin of the swim bladder, turns sharply to the right; it then curves to the left, gradually at first, then more abruptly beneath the liver, again curving sharply to the right at the pylorus to emerge as the intestine from beneath (dorsal to) the liver immediately caudad to the place where it disappeared. Thence it passes in a direct course to its point of fusion with that of component A. The liver, about the size and shape of that of component A, lies ventral to and covering the pyloric bend, its largest part extending some distance to the left of the gut toward the ventral wall of the common abdominal cavity. It sends an elongate, finger-like process cephalad and to the right over the ventral surface of the cardiac stomach. The swim bladder emerges from the gut on the right side and keeps this position with reference to the stomach and the first part of the intestine to a point near the lower margin of the liver, where it terminates.

No. 5 (figs. 4 and 5). This specimen also shows inversion of situs viscerum in component B. The vertebral columns are separate to a point just posterior to the dorsal fins, where they become fused. The caudal fin is doubled. Component B has an abnormal head, smaller than that of A and possessing no eyes and only a rudimentary mouth. Fusion of the intestines occurs just before exit from the abdominal cavity. The viscera of the autosite (component A) are in normal situs, only slight distortion due to crowding being noted.

The curvatures of the gut of the parasite (component B) are apparently reversed, the stomach first curving to the left and ventrally, then slightly dorsad and to the right. Just posterior to the origin of the swim bladder the greater curvature sweeps



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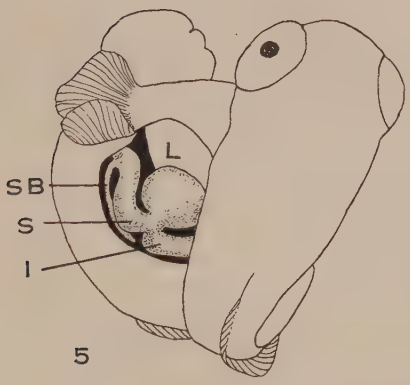

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Fig. 4 Left posterolateral view of specimen no. 5, showing arrangement of of the viscera in the autosite (component A). The situs viscerum is normal. Abbreviations as in fig. 1.

Fig. 5 Right posterolateral view of the same monster shown in fig. 4, showing the viscera of the parasite (component B). The situs viscerum is reversed. Abbreviations as in fig. 1.

posteriorly to the left and slightly ventrad, turning abruptly dorsad at the pylorus to meet the intestine. The liver is discoid in shape and lies to the left of the pylorus. The swim bladder lies dorsolateral to the stomach on the right side.

No. 6. Fusion of the vertebral columns takes place just posterior to the dorsal fins. Component A shows normal development and situs viscerum, but component B is much smaller, possessing no eyes or nares, and only a rudimentary mouth. Its viscera, however, are well developed, but show such great distortion that it is impossible to locate the primary curvatures with exactness. The liver of A, placed to the right of and ventral to the pylorus, is normal in position though slightly abnormal in shape. Its duct enters the gut posterior to the point where it is joined by the intestine of component B.

The gut of the parasite (B), on appearing in the abdominal cavity, passes transversely across it, then makes a sharp turn caudad and to the left, lying in contact with that of the autosite. At the pylorus it makes a slightly wider turn to the right and very shortly fuses with the pyloric stomach of component A. The liver of B lies to the left of the pylorus close to the dorsal wall, its duct enters the gut before the latter's fusion with that of component A. The swim bladders of both components lie in their normal positions with reference to the stomachs.

No. 7. Fusion of the vertebral columns takes place just posterior to the dorsal fins; more caudad there is a single body and tail. Component A is a very small parasite on component B, the head being only about half the normal size and possessing no eyes, nares, or mouth. The intestinal tracts of the two components are separate throughout, the intestine of the parasite being the more poorly developed and opening dorsal to that of the autosite into the single anus. The situs viscerum of both components is normal, the viscera of the parasite being much distorted anteriorly because of lack of space and considerably underdeveloped posterior to the pylorus.

No. 8. The heads of both components are of the same size and entirely separate to a point just posterior to the gill region. The vertebral columns are separate anterior to the dorsal fin, and both pairs of pectoral fins are present. The gut of each component presents curvatures indicating normal situs, though a considerable degree of distortion is shown, particularly in that of component B. The two fuse near the pyloric ends of the

stomachs. The liver appears as a single bilobed mass lying on (ventral to) the point of fusion of the gut of the two components. The larger lobe is somewhat quadrangular in shape, lying ventral to and to the right of the stomach of component A. The smaller, more anterior lobe of the liver mass extends anteriorly in the midline of the monster, filling the interval between the two stomachs. This anterior lobe apparently represents the liver of component B, and the posterior that of component A. The swim bladders were not found, having probably been destroyed in dissection.

No. 9. Fusion of the vertebral columns takes place just anterior to the dorsal fin (which is doubled). The head of each component is subnormal in size, though the two are approximately equal, that of A showing only a rudimentary lens on the left side, with no external trace of an eye on its right. Fusion of the two alimentary tracts occurs at the pylorus. The curvatures of the gut of both components are normal, but both livers and the swim bladder of B are displaced. The liver of A lies transversely across the ventral side of the first part of the common intestine; that of B, the smaller of the two, lies to the left of and caudad to the greater curvature of the stomach. Its duct, however, can be traced across the ventral surface of the gut to its normal point of communication with it. The swim bladder of A is in its normal position, while that of B appears on the right side of the stomach soon after its origin from the dorsum of the gut.

No. 10. The doubling of the vertebral column in this specimen persists posteriorly to a point midway between the tip of the midbrain and the anterior limit of the dorsal fin. The left and right pectoral fins of A and B, respectively, are coalesced to form an abnormal doubled structure, fused proximally, but distinguishable as two distally. The two heads are normal and of equal size. The cardiac ends of the stomachs of the two components rapidly converge on entering the abdominal cavity so that for the greater part of their length they lie side by side with a very narrow interval between them. Near the pylorus they fuse, and caudad to this point the intestinal tract is common to the two components. The curvatures of the gut indicate a

normal situs. The liver is compound, rather larger than a normal single organ, and is placed transversely across the posterior part of the abdominal cavity. It bears two small, obtuse lobules on the right border, overlapping ventrally the pyloric stomach of component A, and one smaller acute lobule extending cephalad between the stomachs. The swim bladder of component A is not demonstrable, that of B emerges from beneath the stomach to lie in the interval to the left of and paralleling the anterior lobe of the liver mass.

No. 11. The vertebral column is doubled anterior to a point midway between the anterior tip of the head and the dorsal fin. The head of component B is set at almost a right angle from the left side of the gill region of A, and in consequence does not extend so far anteriorly. The left eye of A and the right eye of B are lacking. The pectoral fins of the contiguous sides are fused and are smaller than a normal fin. The gut of component B makes a sharp turn caudad as it appears in the abdominal cavity, extending posteriorly to meet and fuse with that of component A in the pyloric region. From this point on the gut is single. The curvatures of both indicate a normal situs. The compound liver is an abnormally small organ placed slightly ventrad, caudad and to the right of the point of fusion of the digestive canals. The swim bladder of A passes posteriorly in the normal position at the left of the stomach, crossing it dorsally where the stomach makes the final bend before fusing with that of B, and extending still further posteriorly under cover of the liver. The position of the swim bladder of component B was not determined.

No. 12. The vertebral column is single posterior to a point midway between the anterior end of the head and the dorsal fin. The head of component B is smaller than that of A and lacks eyes. Fusion of the gut of the two components takes place at the region of the pyloric stomachs. The situs viscerum B is normal, though the liver is displaced to the left side of the abdominal cavity. The gut of component A presents curvatures of a type almost exactly mirror-imaging those of B. They are, however, not typical and the situs is not clear. The liver,

perhaps in part fused with that of B, shows only as a small mass situated between the two stomachs. The swim bladders were not found.

No. 13. Fusion of the bodies of the two components takes place immediately posterior to the gills. The head of B is normal, that of A is smaller and lacks the left eye. The viscera of the monster show considerable distortion, but the situs of component B is obviously normal. The stomach of component A extends caudad to fuse with that of B in the pyloric region, situs indeterminate. The liver is single and situated on the extreme right side of the abdominal cavity ventral and to the right of the pyloric region. The swim bladders were not visible.

No. 14. Only the head and three cervical segments are doubled, fusion taking place in the vertebral columns a very short distance posterior to the base of the skulls. Both heads are normal and equal in size and development. The stomachs of the two components lie very close together and fuse in the middle of the cardiac regions. The curvatures are apparently normal. The livers are fused and the mass of liver tissue lies ventral to the pyloric bend. The swim bladders are normal in size and position, that of A being hidden from view ventrally by the stomachs and pylorus.

No. 15 (fig. 6). The head of the parasite (component A) is hardly more than a bud from the right side of the anterior trunk region of the autosite, the proper vertebral column being very short. It is almost entirely devoid of recognizable organs, only small rudiments of gills being visible. The guts are fused in the region of the pyloric stomachs. That of the autosite presents normal curvatures, while the parasite shows them slightly reduced and apparently mirror-imaged. The stomach of A passes caudad with very gentle curves first to the left, then a longer one to the right, followed by a sharp turn toward the left to the point of fusion with that of B. A single liver mass (possibly compound) is present, situated on the right side of the abdominal cavity just lateral to the pylorus. It is about the size of a normal single organ. The swim bladders of both components are seen protruding from beneath the right side of the cardiac

stomach of component A, the one from B obviously crowded out of position by yolk, while that of A lies on the right side of the stomach throughout its course.

DISCUSSION

The asymmetry relations in the viscera of the double trout examined in this study bear out in general the view set forth by Morrill ('19) that the correlation between the amount of external doubling and the occurrence of situs inversus viscerum

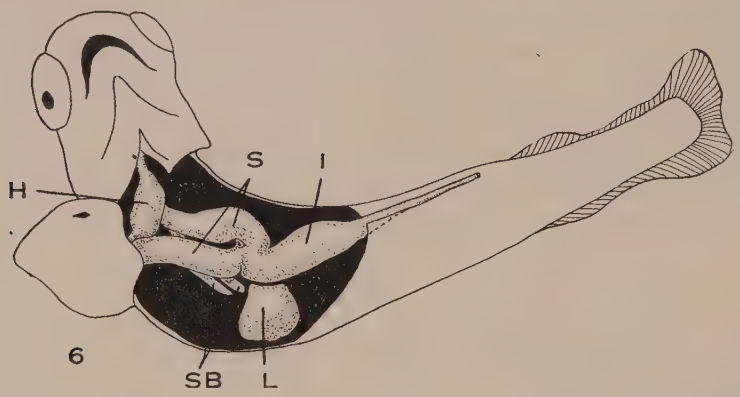

Fig. 6 Ventral view of specimen no. 15. Component A is of the parasite type, B the autosite. Situs B is normal. The gut of A mirror-images that of B. Inversion is not certain; the effect may be due to torsion. Abbreviations as in fig. 1.

is not at all precise. In table 1 the monsters are arranged according to their degree of external doubling. It will be seen that those whose vertebral columns are fused anterior to the dorsal fin and those which are nearly separate show at best only doubtful mirror-imaging. It is further noted, as was also found by Morrill, that not all the animals falling within the limits of doubling apparently most favorable for transposition of the viscera show this phenomenon. Thus there are indications that the reversal of asymmetry is not a necessary consequence of any condition of doubling, but may or may not occur, depending on some other factor capable of operating within these limits.

Column 5 of the table shows the order in which the specimens in this series appear when arranged according to the degree of internal doubling. This indicates a similar condition of correlation to that shown for external doubling, mirror-imaging only occurring when the digestive tracts are fused at some point be-

TABLE 1

Showing trout embryos arranged according to degree of external doubling

NUMBER	SITUS A	SITUS B	REGION OF FUSION VERTEBRAL COLUMN	ORDER OF INTERNAL DOUBLING	REGION OF FUSION OF GUT
1	Normal	Normal	Base of tail	2	Just anterior to anus
2	Normal	Normal	Posterior to dorsal fins	5	Between pylorus and exit from body cavity
3	Reversed	Normal		6	
4	Normal	Reversed		3	
5	Normal (autosite)	Reversed (parasite)		4	
6	Normal (autosite)	Normal (parasite)		7	Irreg. — pyloric stomach with intestine
7	Normal (parasite)	Normal (autosite)		1	Separate
8	Normal	Normal	Anterior to dorsal fins	12	Pyloric stomach
9	Normal	Normal		9	At pylorus
10	Normal	Normal	Half way midbrain to dorsal fin	11	
11	Normal	Normal	Half way snout to dorsal fin	8	
12	Indeterminate	Normal		14	Pyloric stomach
13	Indeterminate	Normal	Just posterior to gills	10	At pylorus
14	Normal	Normal	Ca. 3rd cervical segment	15	Midcardiac stomach
15	Reversed ? (parasite)	Normal (autosite)	Near base of skull	13	Pyloric stomach

tween the pylorus and the exit of the intestine from the abdominal cavity. The table clearly shows that there is no accurate correspondence between the degree of external and internal doubling. Spemann and Falkenberg ('19) find that situs inversus occurs in separate twins produced by constriction of the embryo at blastula or gastrula stage, and in the light of this it cannot be

said that mirror-imaging is in any way dependent on the degree of doubling, external or internal.

It is not in point to discuss in detail at this time the theories of the causes of situs inversus, and the reader is referred to Morrill ('19), pp. 275–81, for a concise review of the recent literature. Pressler ('11), working with Spemann's material, found situs inversus in larvae of Rana esculenta and Bombinator, which had had a portion of the medullary plate and the subjacent endoderm turned end for end in the neurula stage. Spemann ('18), working also with Bombinator, has shown that situs inversus can be obtained by inversion of a small bit of ectoderm and endoderm in the region of the future medullary plate at the end of gastrulation, but inversion of the ectoderm alone at the beginning of gastrulation has no effect. Spemann and Falkenberg ('19) found in the twins and double monsters of Triton produced by constricting the developing embryo at the gastrula, blastula, or even earlier stages, that approximately half of the right twins (corresponding to component A) showed situs inversus, the others being normal or of indeterminate situs. Spemann, in discussing the theories of the causal factors in situs inversus, considers a fundamental inherent bilateral asymmetry in the egg. He suggests as a possible interpretation of the occurrence of mirror-imaging in the right twin after a cut in the midline of the embryo, that the primary asymmetry existing in the right half of the gut anlage may be reversed in some such manner as is that of certain asymmetrical crystals after injury. He considers that this explanation, which was put forward by Přzibram to account for reversal of asymmetry in a limb regenerated from a proximally directed wound surface, may be at least partially applicable to the phenomena which result in the establishment of situs inversus viscerum in the right twin. The possibility of reversing the microstructure of crystals by cutting seems to him highly suggestive in accounting for mirror-imaging in the gut, as inversion of the microstructure of the anlage of one twin would, in the course of subsequent normal development on this basis, produce situs inversus, just as normal development from a normal (not inverted) micro-

structure would result in normal situs. However, in view of the fact that constriction of an embryo of practically any age up to the completion of gastrulation may result in situs inversus viscerum of one of the twins so produced, Spemann considers it more probable that asymmetry reversal in the viscera is brought about as a direct effect of the injury rather than an inversion of the fundamental microstructure. Structural deficiencies are observed to preponderate on the operated side in such experimental animals and the bodies are often definitely bent toward this side. This shows there is a marked effect from the wound, and this influence may be sufficient to transpose the relations of the viscera without any molecular rearrangement in the cellular make-up. The exact manner in which these fundamental changes are brought about remains for future investigations to determine. Spemann suggests that breeding animals with situs inversus may help in clearing up this confusion.

Aside from the fundamental problem of the causes of symmetry reversal, another point deserves mention. Morrill ('19) states that it is component A which shows the mirror-imaging, if inversion is present, component B always being normal. Spemann, in the work mentioned above, describes an exceptional case showing mirror-imaging of the heart of the left twin; the situs of the gut of this twin was not clear. There was also mirror-imaging in the gut and heart of the right twin of this pair. He states that situs inversus almost never occurs in the left twin. In my cases no. 4 and no. 5 we have two more examples of inversion in the left twin (component B). Spemann, in explaining the inversion of situs cordis which he found in this left twin, states that it is probably due to an influence other than that of the operation, affecting the heart anlage from the outer side. In support of this view he cites cases of his own which show defects of the limbs, gills, etc., on the side of the body away from the cut, which have resulted from stimuli not connected with his operative procedure. He also brings up the experiments of Dareste ('77), who produced situs inversus ('l'hétérotaxie') in chicks by the application of excessive heat to the left side of the developing embryo, as corroborative of this point of view.

This work was later repeated by Warynski and Fol ('84) with similar results. It seems improbable that the extensive conditions of inversion found in the two double monsters no. 4 and no. 5 of this study could be due to the effect of some adverse external influence applied in just the right region and at precisely the right moment. As is shown by figs. 2, 3, 4 and 5, there are no unilateral structural abnormalities in either of these cases to be related to retarded or accelerated growth. Both components of no. 4 are externally normal, and though component B of no. 5 is of the parasite type, it is at least bilaterally symmetrical. Again, it is stated by Morrill that in his specimens of the autosite-parasite type, where one component is considerably larger and better developed than the other, mirror-imaging never was found in the parasite, no matter on which side of the monster it was situated. Here it is necessary to call attention to specimen no. 5 (and possibly no. 15), in which it is the parasite which shows the inversion. Interpreting situs inversus according to the theory set forth by Spemann, it is difficult to see why the relative size of the twins should influence the occurrence of mirror-imaging. Indeed, if there were an effect, one might expect that the parasite, showing the poorer development, would more easily be affected than the autosite.

At present the ultimate cause of situs inversus is still hypothetical; the theories which have been advanced for its explanation are suggestive, but not conclusive. There are indications of a general correlation between the degree of doubling and the occurrence of mirror-imaging in the viscera, but since separate twins may also show situs inversus, the significance of any such correlation is very doubtful. Situs inversus may occur in either twin, though the large predominance of rights over lefts in this respect indicates that for some reason the two sides are not equally susceptible. Twins of the parasite type may show situs inversus. It is evident that more data and further experiments along these lines are necessary before a true solution of the problem can be reached.

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Reprinted from THE JOURNAL OF EXPERIMENTAL ZOOLOGY, Vol. 34, No. 2, October, 1921

THE RELATION OF THE PARS INTERMEDIA OF THE HYPOPHYSIS TO PIGMENTATION CHANGES IN ANURAN LARVAE

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FOUR TEXT FIGURES AND TWO PLATES

The experiments recorded here were begun by the writer in April, 1920, at the Biological Laboratory of Princeton University and some of the principal results mentioned in the present paper were obtained at that time. The experiments were discontinued during the summer to be resumed in September at this laboratory on a much larger scale than was possible before. The earlier experiments were confined to transplanting the anterior and intermediate lobes of the hypophysis of adult Rana catesbeiana into immature larvae of the same species, i.e., the grafts were homoplastic. The pars nervosa and pars tuberalis of the gland were not taken into consideration at that time.

In the earlier work it was observed that transplantation of the anterior lobe of the anuran hypophysis into year-old larvae averaging 85 mm. total length induced a slight increase in the body growth of the animals and marked acceleration of limb growth. In two individuals the fore legs appeared, and there were other evidences of metamorphic changes within thirty days from the date of engrafting the glandular tissue. The majority of the larvae did not go so far as this before the experiments were abandoned. Transplants of the anterior lobe of pituitary were also made on larvae averaging 50 mm. total length, without limbs. The growth rate increased markedly in these younger animals, and there was also rapid growth and differentiation of the hind limbs though the metamorphic changes were not nearly so marked nor so rapid as following transplantation of the pituitary

into older larvae. These results are interesting when it is recalled that under normal conditions the larvae of the bullfrog require approximately two years to complete metamorphosis. Fully differentiated legs do not appear usually until the larvae are almost full-grown in regard to size and over a year old. Hence, when hind limbs are induced to grow to a length of 14 mm. in immature tadpoles of 50 mm. total length by transplantation of the anterior lobe of the hypophysis, and such growth occurs within forty days, it is readily seen that the changes induced are indeed marked. The effects upon metamorphosis following transplantation of the thyroid of adult frogs are very much more rapid, and more marked in every feature of the reaction than those following transplantation of the anterior lobe of the pituitary. Grafts of the anterior lobe "activate" the physiologically inert gland of immature larvae.

These experiments on the transplantation of the anterior lobe of the hypophysis and the thyroid gland are not discussed in the present communication, and are mentioned here in order to show that my experiments along this line, together with those recorded here, were begun simultaneously with and independently of the closely similar work of Prof. B. M. Allen, of the University of Kansas. Professor Allen has recently published a brief statement of his results in Science (vol. 52, no. 1342, 1920). Our results are in essential agreement, differing merely in such minor matters as technique, frog species used, and whether homo- or heteroplastic transplants were utilized.

MATERIAL AND METHODS

Allen used normal, pituitariless and thyroidless tadpoles of Rana pipiens and made use of homoplastic grafts only; my own material consisted of the neotenous larvæ of the bullfrog with glandular material from several frog species; i.e., the grafts were both homo- and heteroplastic in nature.

The tadpoles used, in the majority of the later experiments, were caught in the vicinity of New Haven and all came from the same pool. The average size was 55 mm., from snout to tip of the tail; most of the animals had minute hind-limb buds. Dur-

ing the course of the experiments, Elodea and other aquatic plants were used as the source of food supply. Before operating, the animals were anaesthetized in a weak solution of chloretone, and after all movement had ceased were transferred to wet towel paper, ventral side up. A small transverse slit through the body wall and peritoneum was made with dissecting scissors, just lateral to the median line above the mesonephros. The glandular tissue to be transplanted was then shoved through the opening with a blunt needle back toward the median line of the body. In many cases the tissue was transplanted in the abdominal lymph sacs. Either method is satisfactory and equally simple. The mortality resulting from such operative procedure is practically negligible. It is a rather striking fact that an operation as severe as an incision into the body cavity should be followed by such rapid recovery and so few fatalities. There is, however, practically no loss of blood, and the wound is very soon closed by a lymph clot that serves to hold the transplanted tissue in place and prevents other foreign substances from entering the body cavity. Recovery from the effects of the chloretone is rapid, and apparently no part of the procedure causes any harmful results. Normal animals used as check controls were treated in a similar way with engrafted brain and muscle tissue instead of glandular substance.

The glandular material was taken from adult frogs of three species, Rana catesbeiana, Rana pipiens, and Rana clamitans, several young specimens of the latter species were also employed.

The hypophysis of an adult frog is readily dissected out by first severing the head from the body just behind the medulla, and then with a pair of sharp-pointed dissecting scissors clipping away the floor of the brain case and exposing the gland. I have found it expedient to cut out the hypophysis and tuber cinereum as a unit in my later experiments, on account of the intimate relation between the pars tuberalis of the hypophysis and this portion of the brain floor.

It is well known to all recent investigators of the pituitary that the gland consists of four parts or lobes, as they are sometimes called, each portion distinct from the others both ontogenetically and histologically. The epithelial part of the pituitary

or that portion which takes origin from the wedge-shaped invagination of cells from the inner layer of oral ectoderm in the early embryo, differentiates during the course of development into three parts: the anterior lobe, the pars intermedia, and the pars tuberalis, first recognized by Tilney ('13) as a distinct portion of the gland. The remaining lobe of the gland, the pars nervosa, is derived from the tip of the infundibulum. It is not within the scope of this paper to discuss at length the anatomical relations of these parts of the hypophysis in adult Anura, especially in view of the recent excellent paper by Atwell ('19). In figure 1 is shown a ventral view of the hypophysis of the bullfrog illustrating the positions of the various lobes.

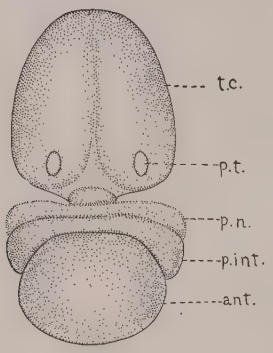

Fig. 1 Ventral view of the hypophysis of an adult Rana catesbeiana. *t.c.*, tuber cinereum; *p.t.*, pars tuberalis; *p.n.*, pars nervosa; *p. int.*, pars intermedia; *ant.*, anterior lobe.

The pars tuberalis is seen to consist of two plaques which underlie the lateral thickened portions of the tuber cinereum (infundibulum of many writers, though it is doubtful if the lateral thickened portions of this structure can properly be regarded as such). It is readily seen from the diagram that this portion of the hypophysis is entirely separate from the remainder of the gland.

The anterior lobe is the most conspicuous of the remaining parts of the gland, and paradoxically, instead of being the most anterior as its name implies, it is the most posterior part of the hypophysis in the frog. Its outline is oval, and it is a delicate pink in color, due to the presence of numerous blood vessels.

The pars intermedia is a transverse band of tissue with bulging lateral terminations, closely applied to the anterior lobe. Posteriorly it conforms so closely to the shape of the pars nervosa that it is difficult to make a clean-cut dissection of the two parts under the microscope. The texture and color of the tissue of the two two lobes differ to such an extent, however, that is is easy to distinguish them.

OBSERVATIONS

Forty-seven larvae, light greenish-yellow in color, were engrafted intraperitoneally with the pars intermedia of the hypophysis of the three species of Rana mentioned. Sixteen hours after the operation, the experimented animals were distinctly darker than the controls, and at the end of forty-eight hours they were nearly black. The controls, engrafted with brain and muscle tissue, retained the normal light-greenish yellow color (fig. 2). The change in color following the operation is very striking, indeed, on account of the color contrast between control and hypophysis-grafted tadpoles, and also because it is so uniform. Any possibility of the environment's playing an active rôle in the reaction was ruled out by the controls, although it is quite true that chloretone tends to cause slight expansion of the melanophores. It was observed that after anaesthetizing the control tadpoles in chloretone the animals turned somewhat darker ten to twenty minutes afterward; this condition lasted but a short time, after which the larvae resumed their normal coloration. The reaction was not at all comparable to the striking effects following transplantation of the pars intermedia (fig. 2).

The darkly pigmented condition persists in the engrafted animals for varying periods, but in none did it last longer than forty days. It has been my experience that the larvae engrafted with the glandular tissue from adult frogs of different species, i.e., heteroplastically grafted, are the first to return to normal coloration. Animals homoplastically grafted tend to retain the dark color for longer periods, though this is certainly not an invariable rule. In the case of heteroplastic transplants, twenty-six days was the maximum and ten days the usual period for the retention of the

dark color. After this there is a gradual (but in some instances a very abrupt) change to the normal greenish-yellow coloration. The average time required for animals homoplastically grafted to regain their usual color is twenty-six days from the date of the operation. The change from almost black to yellow is usually a gradual process, parts of the tadpole body turning yellow, other portions retaining the dark color, thus giving the animal a sort

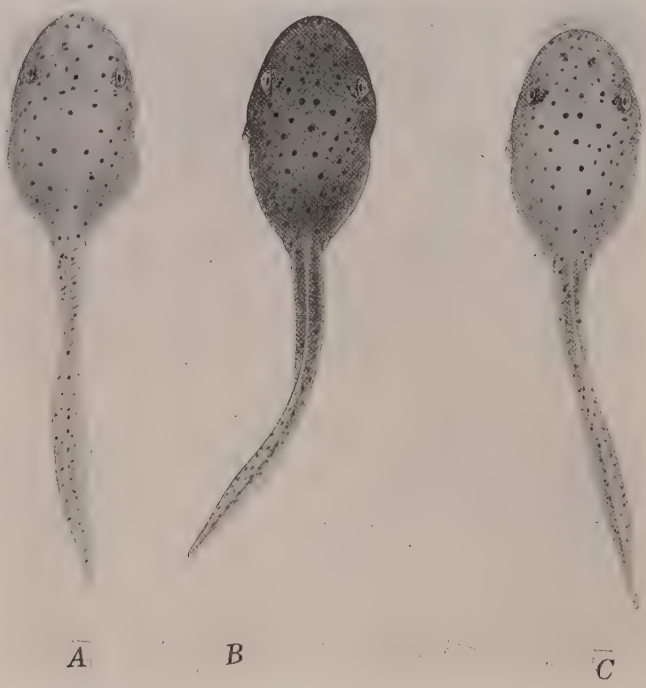

Fig. 2 A, normal control; *B*, engrafted larvae; *C*, engrafted animal following resorption of the graft.

of mottled effect. Three cases were observed of larvæ resuming the yellow color overnight from a deeply pigmented condition. In figure 2 the same tadpole is shown darkly pigmented in *B*, and in *C*, twelve hours later, it has changed from dark to yellow.

Histological examination of the grafted tissue of such individuals reveals clearly the reason for the resumption of normal coloration in these tadpoles. The graft has, for the most part,

been resorbed; that is, the glandular tissue has disappeared, and nothing remains of the transplant but a mass of connective tissue and blood vessels. On the other hand, in the black individuals the engrafted tissue always shows the presence of secreting glandular cells. The transplanted tissue of the hypophysis in both hetero- and homoplastic grafts evidently is largely resorbed in bullfrog larvae of 55 mm. total length about twenty-six days or less from the date of transplantation. The transplanted tissue is generally found attached to the peritoneum of the body wall and is much more satisfactorily handled for microscopic examination when left attached to a section of the somatopleure than when separated from it. Otherwise, the minute size of the graft renders handling difficult. The implanted pars intermedia very soon becomes invaded with blood vessels from the peritoneum. Allen ('20) failed to state whether or not any of his animals resumed normal coloration following transplantation of the pars intermedia, nor was any histological examination made to ascertain the fate of the graft. A more detailed report by Professor Allen upon this point is awaited with interest.

Several tadpoles, previously darkly pigmented by reason of the engrafted tissue of pars intermedia, but which subsequently resumed the yellow color owing to resorption of the transplant, were reëngrafted. The results were always the same, the animals turned very dark for several weeks, then returned gradually to normal coloration. Aside from the more rapid resorption of the grafted tissue of other species of frogs, it apparently made no difference in my experiments, so far as the results were concerned, whether the transplant was homo- or heteroplastic in nature. This is more or less to be expected in grafts involving endocrine glands, owing to the presence of the physiologically active hormone in the glandular substance transplanted. Even though the graft be resorbed, a sufficient amount of the active hormone is generally obtained to bring about a response on the part of the organism.[1] In a recent series of experiments with

[1] The pigmentary changes are more marked when the pars intermedia and pars nervosa are transplanted together, than when the intermediate lobe is grafted alone. The inclusion of the posterior lobe causes a remarkable shrinkage of the larvae due to the diuretic effect upon the mesonephros and consequent loss of turgor by rapid elimination of water.

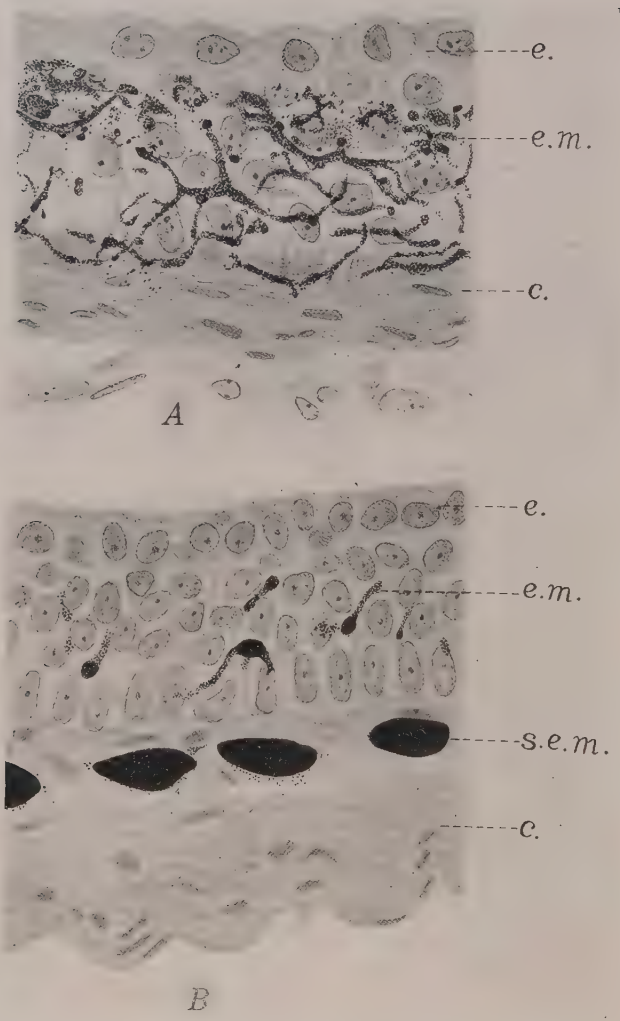

Fig. 3 A, section through skin of engrafted animal. *e*, epidermis; *em*, epidermal melanophores; *c*, stratum spongiosum of corium, containing greatly expanded subepidermal melanophores. B, section through skin of light-colored control. *e.m.*, epidermal melanophores; *s.e.m.*, subepidermal melanophores contracted; *e*, stratum corneum; *c*, corium.

transplanted thyroid glands from animals dead over twelve hours, this point has been well brought out.

A word regarding the various types of pigment cells found in the frog skin may serve in some measure to clarify the discussion to follow. There are several varieties of pigment cells which normally occur in the skin of anurans, and of these there may be distinguished: the melanophores or black pigment cells, leucophores or interference cells, xanthophores, and xantholeucophores or golden pigment cells. Some species of frogs have red pigment.

The melanophores are regarded by the majority of investigators as fixed stellate cells with many irregular branching processes. The pigment is in the form of numerous minute brown or black granules of melanin scattered in the cytoplasm. This type of chromatophore is most abundant on the dorsal surface of the body, and occurs in dense masses in the black spots found scattered over the back and sides of the animal. In sections through the skin fig. 3 *B*) they are found mostly in the superficial layer of the corium just below the epidermis. A somewhat different type of chromatophore is found in the epidermis; it consists of a cell body with two or more simple processes. Such cells usually lie singly in the epidermis (especially in lightly pigmented individuals), though they may occur in darker animals in sufficient abundance to give the appearance of a reticulum. This type of melanophore is said by Hooker ('14) not to be contractile, though the writer is inclined to question this assertion, especially as regards conditions in Rana catesbeiana. On the ventral surface of the tadpole and frog body, these two types of pigment cells are almost absent over considerable areas. The subepidermal melanophores are innervated by motor fibers proceeding along both sympathetic and spinal paths, but, as Laurens ('15) has shown, this type of pigment cell may also respond to direct stimulation without the intermediation of the nervous system. As shown in figure 3*B*, the subepidermal melanophores present, when contracted, the appearance of small rounded densely staining masses.

According to Smith ('20) , the xantholeucophores of the anuran larva form two well-defined layers over the dorsal portion of the

body, each layer being composed of independent cells. The superficial layer lies beneath the epidermal basement membrane, and is separated by a considerable interval from the deeper layer situated just above the corial melanophores. These cells bear guanine and xanthine, and when expanded, give the silvery and iridescent color effects so marked in hypophysectomized tadpoles.

The dark engrafted individuals, when examined under a binocular microscope, showed great expansion of the epidermal and

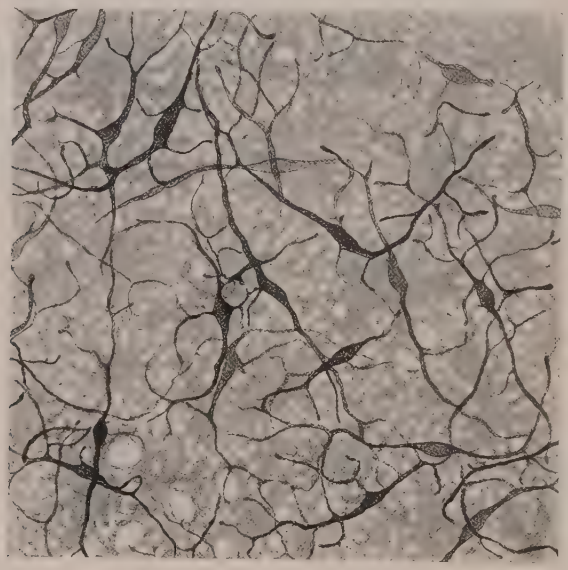

Fig. 4 Surface view of whole mount of skin from engrafted larvae. The two layers of melanophores epidermal and corial are greatly expanded.

subepidermal melanophores of the skin as compared with the normal controls. The degree of expansion of the superficial melanophores of the experimented larvae is striking, the much branched and irregular network of cellular processes apparently forming a dense reticulum. The expansion of the subepidermal pigment cells is so great that an opaque sheet of melanin is found. In the light-colored controls, the melanophores are contracted into dark ovoid masses, and there is no network or reticulum of epidermal melanophores. It is possible, however, to find in any

large culture of bullfrog larvae, where there is unequal distribution of light and shadow, certain individuals with more or less expanded melanophores of both epidermal and subepidermal type. When these dark tadpoles are examined, it is found that the pigment reticulum, though present, is not so dense as in the engrafted animals. In such larvae the dark pigmentation is temporary, and the expansion of the melanophores fluctuates with changes in the evironment. Such is not the case with engrafted larvae, for when they turn dark, the expansion of the pigment cells is permanent under all conditions of the environment, so long as the glandular tissue remains intact and supplies the necessary stimulus to the melanophores (fig. 4).

Not only are the subepidermal melanophores expanded to their maximum extent in engrafted animals, but this expanded condition of the pigment cells apparently extends throughout the body (fig. 10). Examination of the deep-lying melanophores, such as are found in large numbers on the lungs, liver, peritoneum, pericardium, and brain membranes, showed that the effect of the engrafted pars intermedia is not confined solely to the superficial pigment cells of the epidermis and corium. Pigmentary conditions in the deep-lying organs are not so striking and clear-cut as in the skin, because of the fact that in normal larvae the melanophores of these structures are usually in a more or less expanded condition. If, however, the lungs of normal and experimented tadpoles are dissected out and used as a basis for comparison, it is readily seen that the degree of expansion of the numerous melanophores on these organs of the engrafted animals is greater than that of the normal controls.

These observations are directly in line with those of Allen ('17) on hypophysectomized tadpoles. He found that the animals deprived of the epithelial portion of the hypophysis show a contracted condition of the melanophores throughout the body. That is, when the pars intermedia is extirpated, the pigment cells all over the body contract and remain in this condition permanently. Smith, however, finds in his careful study of albinism in tadpoles deprived of the buccal portion of the hypophysis that in young animals the subepidermal melanophores are contracted

as Allen stated, but in older animals the corial sheet of pigment cells appears much as in normal larvae and persistent contraction of the melanophores does not occur. The silvery appearance of the pituitariless animals is due primarily to reduction in number and melanin content of the epidermal melanophores and the persistent expansion of the layers of xantholeucophores.

The contraction and expansion of the melanophores throughout the body following hypophysectomy in the first instance and transplantation of the gland in the second, is to be expected. It would be odd, indeed, if only the superficial melanophores were affected by the hormone, or activating substance of the pars intermedia.

Fixed and stained preparations of the skin and other tissues were employed as a means of checking conditions observed in the living tadpole. Whole mounts and sections of the skin were both used, the skin being taken from the dorsal surface of the body. Because pigmentary conditions are more readily followed in the skin than in other tissues, the discussion is chiefly confined to changes occurring in the epidermal and subepidermal melanophores.

It was observed in sections through the skin and in whole mounts of skin from the experimental animals that the subepidermal pigment cells were greatly expanded, their processes extending like branches of a tree through the stratum spongiosum of the corium. Expansion is so great that the processes of the different cells interlace to form a very dense pigment sheet. In figure 3*A* is shown a section through the skin illustrating this point. The much-branched melanophores are shown cut in various planes by the microtome knife. Figure 3*B* shows the condition presented by the light-colored control larvae. Here the subepidermal melanophores are shown as much contracted masses lying in the corium. Transverse sections are not so good for illustrating expansion and contraction of the melanophores as are surface views of the integument in which the entire cell appears instead of portions of it. Figure 6 shows a photomicrograph of the melanophores of an engrafted animal from a surface view of the skin, or rather a section cut obliquely in such a manner that practically a surface view is obtained, and in figure 5 is shown a

similar view of a normal (light-colored) animal. The difference in the degree of expansion of the pigment cells is obvious. Figure 10 shows the expansion of the deep-lying melanophores in the meninges of the brain in an engrafted specimen.

As previously mentioned, Hooker ('14) states that the epidermal melanophores are non-contractile, and although this may be true of the form with which he worked, it is apparently not the case with young Rana catesbeiana larvae. In skin preparations of this species, both in whole mounts and in sections, it is evident that in light-yellow individuals the expanded epidermal type of melanophore is very scarce and difficult to find. This is exceptionally well shown in whole mounts of the skin. The subepidermal cells are found to be contracted and present the appearance of black balls, with little indication of cellular structure. On the other hand, whole mounts of the skin of darkly colored engrafted tadpoles show two distinct layers of chromatophores, a superficial and a deep, lying at different levels. The upper layer or epidermal melanophores form a definite network, with the cell processes of neighboring cells in contact, thus giving the appearance of a recticulum (fig. 6, also fig. 4). The deeper layer of pigment cells (subepidermal) are greatly expanded and present the familiar 'mossy' appearance characteristic of this phase of the cell. It seems to the writer that, unless the epidermal melanophores possess the power of contractility, it is otherwise difficult to explain why light-colored animal show so few of these cells, while in engrafted individuals they are not only very numerous, but their processes give the appearance of an anastomosing network. In sections through the skin, similar conditions are seen; the epidermal melanophores are numerous, with long cell processes in deeply pigmented engrafted larvae, but very sparsely scattered in the epidermis of the light-colored controls. Judging from conditions in my material, it seems that these pigment cells are more or less contractile, though evidently not to the extent that the larger, subepidermal melanophores are. They apparently increase in number, or at any rate become visible with elongate processes in engrafted animals, thus playing an active part in the color change following transplantation of the pars intermedia.

Laurens, working on Amblystoma larvae, states that the epidermal melanophores of this form expand and contract, and hence appear to play an active rôle in pigmentation changes. The recent work of Smith likewise indicates that this type of pigment cell is contractile.

In this connection it is interesting to note that following hypophysectomy the resulting albino larvae appear to lose their epidermal melanophores to a large extent, and according to Atwell ('19) the few that remain contain only a small proportion of the normal number of pigment granules found in similar cells of the control larvae. Allen ('17) thinks that following removal of the pituitary, the epidermal pigment cells migrate to deeper positions in the animal, a conclusion with which Smith disagrees. However this may be, transplantation of the pars intermedia causes the appearance of large numbers of this type of melanophore in the skin of the bullfrog tadpole, though there can be little doubt that the dark color of the engrafted animals is due chiefly to the subepidermal melanophores. It is highly improbable, as Smith has shown, that the corial chromatophores play an important rôle in the picture of albinism resulting from hypophysectomy, no matter whether they are contracted or expanded, because they are completely masked by the broad and persistent expansion of the layers of xantholeucophores above them.

Transplantation of the pars intermedia brings about contraction of the xantholeucophores, thus enhancing the 'darkening' of the larvae. In the present discussion we are not concerned with this type of pigment cell.

DISCUSSION

The rôle of the hypophysis in producing pigmentary changes in anuran larvae has received considerable attention since the publication of the work of Smith ('16) and Allen ('17) on hypophysectomy in the frog embryo. Both of these investigators, working independently, succeeded in ablating the epithelial anlagen of the hypophysis at the time of its first appearance as a wedge-shaped invagination of cells from the oral ectoderm. Within a few days following the operation the tadpoles lose their

normal dark coloration and turn silvery white, or, according to Smith, they become albinos. The pigmentary change is characteristic and striking.

Smith and Allen hold somewhat different views regarding the nature of the change in pigmentation following hypophysectomy. Smith considers the silvery appearance of the larvae as being due primarily to a reduction in number and melanin content of the epidermal melanophores, and to persistent expansion of the xantholeucophores. The expansion of the latter gives the tadpole its silvery appearance. He states that the corial melanophores are equally expanded in the albinos and the control animals.

Allen, on the other hand, believes that the albino effect is due to the migration of the epidermal pigment cells to deeper positions in the body and, moreover, that all the melanophores throughout the body are contracted following ablation of the epithelial portion of the pituitary.

Neither of these investigators had any good evidence as to just what portion of the hypophysis is responsible for the change in color following extirpation, for in their experiments three parts of the hypophysis were removed, anterior lobe proper, pars intermedia, and the pars tuberalis. It remained for Atwell ('19) to show that it is the lack of the pars intermedia that is responsible for the albino appearance of the hypophysectomized larvae. He extirpated the hypophysis of the frog embryo and, following the 'silvering' of the tadpoles, placed them in dilute extract of pars intermedia of beef pituitary. The animals soon underwent a striking color change from silvery to dark, in the latter condition closely resembling normal tadpoles. When returned to fresh water the darkened larvae regained the silvery appearance.

Atwell interpreted his results as indicating that the 'silvering' of the hypophysectomized larvae was due to a prolonged and sustained contraction of the pigment-bearing cells, owing to loss of the pars intermedia. Smith, in his recent monograph, puts a different interpretation on Atwell's work He confirms Atwell's observations that the corial melanophores expand in solutions of pars intermedia, but he noted further that not only do the larvae become darker, but they almost completely lose their metallic

iridescent appearance so characteristic of the picture of albinism. That is to say, the xantholeucophores are contracted and the silvery appearance is lost, the animal appearing dark like normal tadpoles. Hence, the picture of albinism is not so much due to contraction of the corial melanophores as to expansion of the overlying layers of xantholeucophores. This interpretation of Smith's has recently been controverted by Atwell.

In a recent communication ('20) Allen has reported a very brief summary of his results with transplanting the pars intermedia of the adult frog. He says: "Normal tadpoles into which the intermediate lobe is engrafted become much more darkly colored than the controls, while those which have been made to turn white as a result of removal of the anlage of the hypophysis exclusive of the posterior lobe are made to change back from white to black when the intermediate lobe is engrafted into them."

In view of these experiments and those recorded here, it can be regarded as a well-established fact that in anuran larvae the pars intermedia secretes a substance which exerts a powerful effect upon the melanophores. The absence of the hormone results in permanent contraction of many of these cells, and expansion of the xantholeucophores, its presence in large quantities such as follows transplantation of the glandular tissue, causes maximum expansion of the epidermal and corial pigment cells and contraction of the bearers of guanine and xanthine.

It has long been known that many conditions affect the melanophores of the frog skin; a list of such agencies includes temperature, electric currents, variations in the intensity of light, and many chemical substances aside from hormones. The effect of such stimuli, however, is temporary and lasts only so long as the melanophores are subjected to the influence of the agent. On the other hand, in the case of the hormone of the pars intermedia of the hypophysis, we are dealing with an internal regulating agent of high potency, the effects of which are continuous, and not to any extent influenced by changes in the environment. For instance, engrafted larvae which have turned dark do not respond by color change, to light stimulation, and neither do albino larvae deprived of the pars intermedia. Smith has shown that the cor-

ial melanophores of such larvae expand under the stimulus of light, but the xantholeucophores are not affected, consequently the 'pituitariless' animal remains silvery.

Many of the agents mentioned exert their influence upon the melanophores through the intermediation of the nervous system. In the transplantation experiments recorded here it is probable that the hormone acts directly upon the melanophores themselves by way of the blood stream, and not through the intermediation of the nervous system, though the latter possibility is not ruled out. However, if, as has been clearly shown by Laurens ('15), other agencies are capable of acting directly upon the melanophores, it is very likely that the hormone of the pars intermedia does likewise.

It is interesting to note that the secretion of another endocrine gland, arising as an outgrowth from the dorsal surface of the diencephalon, has a marked effect upon the melanophores of anuran larvae. McCord and F. P. Allen ('17) have clearly demonstrated that the pineal gland of mammalia, when administered in various ways to anuran larvae, brings about contraction of the subepidermal melanophores, thus causing the tadpoles to assume a condition simulating the silvery appearance characteristic of hypophysectomized animals. The effects of this gland are temporary and soon disappear.

Recently the writer transplanted the pineal gland of reptiles (Chelonia) into darkly pigemented larvae of Rana climitans, and obtained rather interesting results. Within thirty to forty minutes following implantation of the gland into the body cavity, the larvae turned distinctly lighter in color, i. e., from a dark brown to a light greenish-yellow. The light color persisted for several hours, when the animals gradually resumed normal coloration. Mammalian pineal-gland substance (desiccated) in form of pills was injected into the body cavity with similar results. These experiments confirm the previous work of McCord and Allen (F. P.) upon feeding mammalian pineal substance to tadpoles, though the color change following implantation or injection of the gland in my larvae was not nearly so striking as that described by these investigators when the tissue is fed.

It has been demonstrated by several investigators that ablation of the pineal apparatus of amphibian embryos apparently has little effect upon the growth and development of the pigmentary system. It should be noted, however, that this gland when extirpated in very young larvæ has the power to regenerate, hence it is possible that it may play a more important rô'e in inducing pigment changes than we think. It is certain that the p neal gland exerts no such marked and continuous power over the melanophore system as does the pars intermedia.

It is interesting to note that the removal of the pars intermedia causes persistent expansion of the xantholeucophores, and that implantation brings about their contraction along with persistent expansion of the melanophores. Here we are dealing with a differential effect upon the pigmentary system of a single gland, maximum expansion of the melanophores when an oversecretion of pars intermedia is obtained, as, for instance, by transplantation experiments, maximum expansion of the xantholeucophores when the pars intermedia is lacking. Taking these facts into consideration, it is possible that instead of dealing with differential effects of a single gland upon the tadpole's pigmentary apparatus, we may be observing the effects of interaction between two internal secretory mechanisms. It seems rather improbable that the mere absence of the pars intermedia is capable of bringing about maximum and continuous expansion of the xantholelcophores, such as occurs in 'pituitariless' frog larvae. It is improbable that the 'absence' of anything could act as a stimulus of the strength required to produce this effect. Is it not possible that the ablation of the pars intermedia has released some other active agent within the body from an inhibitory influence exerted by this portion of the hypophysis? This is only a suggestion, and should be regarded as such.

In this connection it is of paramount importance to know which condition of the chromatophore, the expanded or contracted, is to be regarded as the active or passive phase. If, as assumed by some (Spaeth, '16, and Laurens, '15), the expanded condition of the pigment cell is the passive phase then the persistent expansion of the xantholeucophores following hypophysectomy in the

embryo frog is accounted for, but not the diminution in number and melanin content of the epidermal melanophores. On the other hand, if, as stated by many workers on pigment cells, the expanded condition is the active phase, then we are faced by a difficult problem when considering the albino, for unless the alternative proposition, that some other internal secretory mechanism is at work along with the pars intermedia, is adopted, we must assume that an 'absence' can bring about very positive differential results within a single system.

In conclusion, the writer takes this opportunity to express his obligation to Miss Julia E. Lovett, of the Osborn Zoölogical Laboratory, for the excellent drawings.

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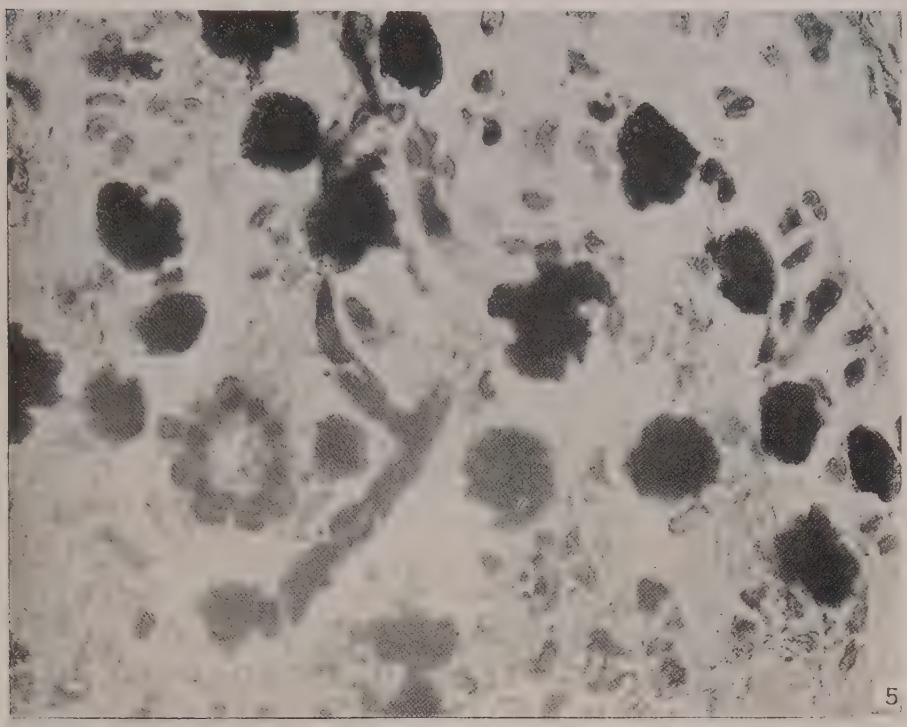
PLATE 1

EXPLANATION OF FIGURES

Microphotographs

5 Contracted condition of subepidermal melanophores of normal larvae. Surface view from whole mount of skin. × 700.

6 Expanded melanophores giving the appearance of a reticulum. Surface view from section cut on bias. × 700.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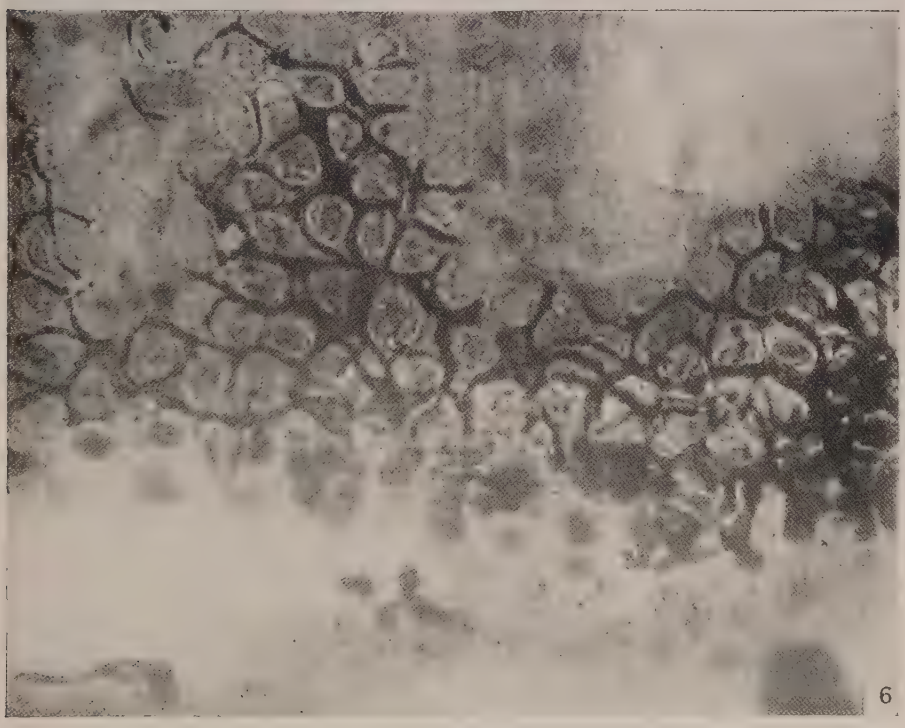
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PLATE 2

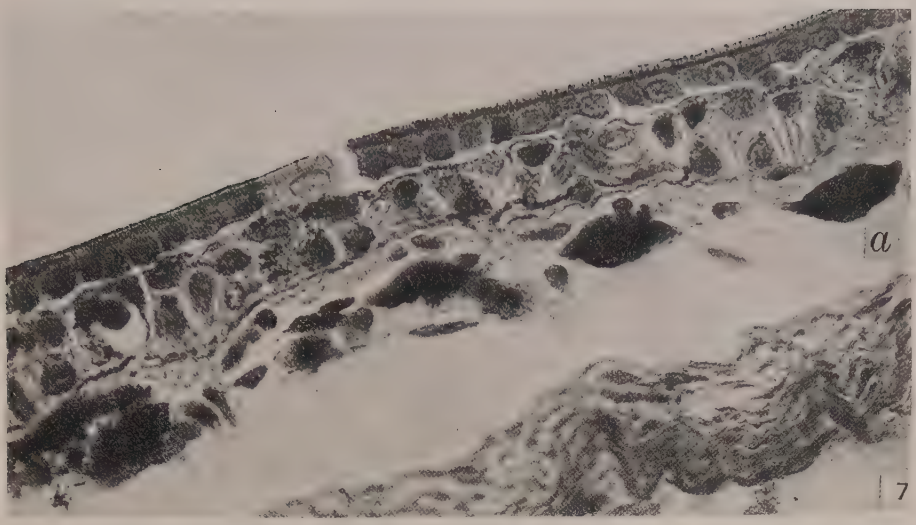
EXPLANATION OF FIGURES

7 Section through the skin of control, showing contracted melanophores at *A*. × 700.

8 Section through skin of engrafted individual. Expanded epidermal melanophore above the subepidermal pigment cells at *B*. × 700.

9 Expanded melanophores in skin of engrafted animal. × 700.

10 Expanded melanophores in meninges of brain of engrafted animal.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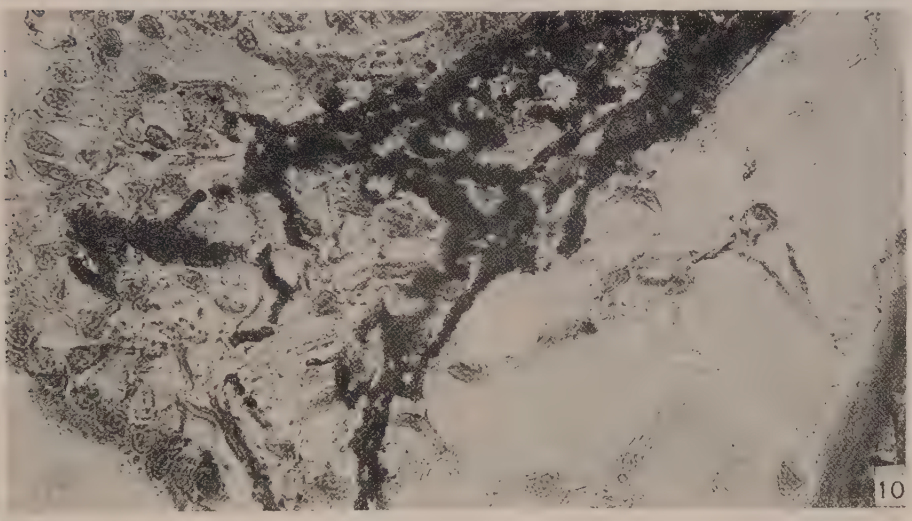
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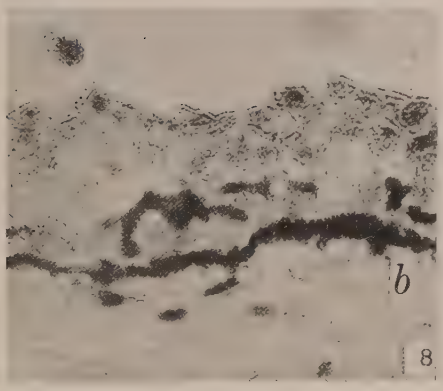
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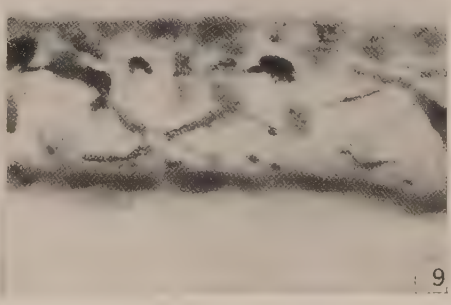
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 17

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOOLOGY, Vol. 34, No. 3, November, 1921

MICRONUCLEATE AND AMICRONUCLEATE RACES OF INFUSORIA

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FOUR FIGURES

The dimorphic condition of the nuclear apparatus of the Infusoria is one of the most interesting specializations exhibited by the group, representing, as is well-known, a segregation of the so-called 'somatic' or trophochromatin from the 'generative' or idiochromatin into distinct bodies, the macronucleus and micronucleus. It has generally been accepted that this differentiation of the nucleus is a diagnostic character of the Infusoria, and, aside from parasitic or aberrant species, the only apparent exceptions have revealed the micronuclei as distinct bodies within the macronuclear membrane during vegetative stages, or emerging as such at the onset of conjugation.

It is true that there are many ciliates in which the micronucleus has not been observed, but naturally such cases are attributed rather to the difficulties sometimes involved in determining these bodies than to their absence. This conclusion has been strengthened by the fact that in micronucleate forms the removal of the micronucleus through degeneration or by experimental means has proved to be the death warrant of the cell. All the evidence indicates that once the chromatin differentiation is established during conjugation or endomixis, the die is cast so far as the macronucleus is concerned. It is destined to play its part during the vegetative life of the cell and to be replaced from the micronuclear complex at the next conjugation or endomixis period.

The recent work of Dawson[1] in this laboratory, however, has reopened one phase of the question. He found in an infusion a race of Oxytricha hymenostoma without a micronucleus and bred it successfully in pedigree cultures for several years. His experiments showed that periodically the animals were in a condition suggestive of conjugation, but merely abortive results followed, such as 'cannibalism,' etc. In brief, Dawson's work demonstrates that a morphologically differentiated micronucleus is not a sine qua non for the life of the individual free-living cell nor for the life of the race during reproduction by division. And, further, it emphasizes the irreversibility of the transformation of micronucleus into macronucleus, since, in his long series of experiments, during the stress, so to speak, of what must be interpreted as abortive attempts to conjugate, micronuclei were not forthcoming.

More evidence indicating the sufficiency of the uninucleate condition for the vegetative life of the cell has just been reported by Landis and by Patten.[2] The former has under culture an amicronucleate race of Paramecium caudatum. Furthermore, he believes that this race can conjugate successfully, and if this proves to be true it will raise a still more interesting question. Miss Patten, of this laboratory, is studying a pedigree culture of an amicronucleate race of Didinium nasutum which arose from conjugating micronucleate individuals.

The purpose of the present paper is to record the discovery of other amicronucleate races, representing Oxytricha fallax, Urostyla grandis, and Paramecium caudatum.

With regard to the technique employed, it will suffice to say that it has involved the use of the several well-established methods for the cytological study of the Infusoria, including those used in our studies on endomixis.[3] Certainly, the results

[1] J. A. Dawson, An experimental study of an amicronucleate Oxytricha. Jour. Exp. Zoöl., 1919, vol. 29, p. 473; 1920, vol. 30, p. 129.

[2] E. M. Landis, An amicronucleate race of Paramecium caudatum. Amer. Naturalist, 1920, vol. 54, p. 453. M. W. Patten, The life-history of an amicronucleate race of Didinium nasutum. Proc. Soc. Exp. Biol. and Med., 1921, vol. 18, p. 188.

[3] L. L. Woodruff and R. Erdmann, A normal periodic reorganization process without cell fusion in Paramecium. Jour. Exp. Zoöl., 1914, vol. 17, p. 425.

are consistent, since, for example, the study of two races of the same species under identical conditions invariably revealed one micronucleate and the other amicronucleate.[4]

OXYTRICHA FALLAX

A specimen of Oxytricha fallax was isolated from a laboratory aquarium on January 28, 1920, and a pedigree culture started by the usual daily isolation method. Standard beef extract was employed as the culture medium.[5] A cytological study disclosed no micronuclei, and therefore the culture was not employed for the purpose intended, but carried on for a thorough examination of its nuclear apparatus and its life-history.

The graph of the rate of reproduction of the lines of this culture is given in figure 1. It exhibits no unusual features, except possibly greater fluctuation in division rate than one usually finds in hypotrichous ciliates when bred under essentially constant culture conditions. The great fall in the rate of division during the fifth five-day period, however, is clearly related to the fact that during the previous five days daily isolations were not made and fresh culture medium was not supplied. The cause of the sudden death of the race on May 4, 1920, at the 246th generation is not apparent, since the vitality as measured by division rate of the previous five-day period was not low. Twice during the life of the culture a few pairs of conjugants were observed in the mass cultures formed of animals discarded from the pedigreed lines at the daily isolations. The conjugants failed to live when isolated, and when preserved revealed no evidence of micronuclei.

In a word, this race consistently failed to reveal a morphologically differentiated micronucleus, though it possessed the potentiality to produce by division at least the number of descendants represented by 2 to the 246th power.

[4] Miss Hope Spencer, of this laboratory, has assisted me in conducting the various pedigree lines and in making permanent preparations. Professor Baitsell oriented, by his plasma method (Proc. Soc. Exp. Biol. and Med., 1921, vol. 18, p. 172), certain animals for sectioning.

[5] L. L. Woodruff and G. A. Baitsell, The reproduction of Paramecium aurelia in a constant culture medium of beef extract. Jour. Exp. Zoöl., 1911, vol. 11, p. 135.

UROSTYLA GRANDIS

On May 4, 1920, a heavy growth of Urostyla grandis was discovered in a laboratory aquarium, and during the following week ten animals were isolated to form ten pedigree lines. These were bred on the standard beef-extract medium which was supplied fresh daily and inoculated with a pure culture of Colpi-

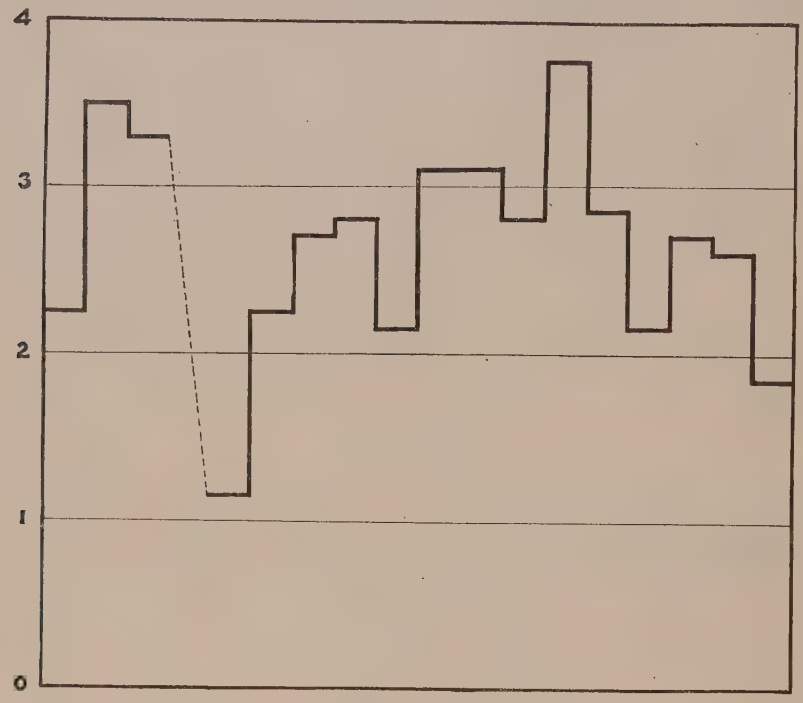

Fig. 1 Graph of the rate of reproduction of an amicronucleate race of Oxytricha fallax. The average daily rate of division of the lines is again averaged for five-day periods.

dium. Urostyla will survive on the bacterial flora which normally develops in the beef extract, but for vigorous growth some small protozoan form must be supplied as food.

Four of the pedigree lines of Urostyla proved to be micronucleate and six, amicronucleate. Accordingly, they were not suitable for the experiments planned, but were continued for varying periods for further cytological study. The ten lines are summarized in table 1.

The graph of the division rate of line Ub which was continued longer than any of the others and accordingly studied most thoroughly is given in figure 2.

TABLE 1

LINE	MICRONUCLEUS	DAYS BRED	GENERATIONS ATTAINED
Ua	Absent	80	40
Ub	Absent	191	128
Uc	Present	22	8
Ud	Absent	33	15
Ue	Present	4	3
Uf	Present	18	9
Ug	Absent	3	2
Uh	Absent	35	10
Ui	Absent	3	2
Uj	Present	27	19

In brief, these ten lines throughout their life bred true with respect to the presence or absence of micronuclei—certain lines (Uc, Ue, Uf, Uj) invariably exhibiting large, easily discernible micronuclei, while other lines (Ua, Ub, Ud, Ug, Uh, Ui) consistently were without micronuclei (cf. figs. 3 and 4).

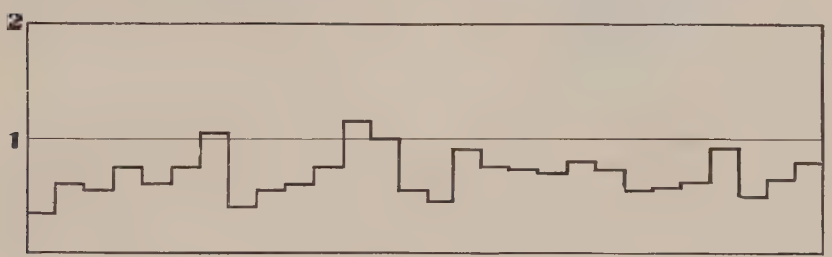

Fig. 2 Graph of the rate of reproduction of an amicronucleate race of Urostyla grandis. The average daily rate of division of the lines is again averaged for five-day periods.

Urostyla grandis is not a common hypotrichous form, and the only time I have ever found it at New Haven was in the mass culture mentioned above. Accordingly, it seems probable that all of the animals of the 'wild' culture were descended from the same stock and that the separation into a micronucleate

and an amicronucleate race occurred while in this culture. If such was the case, it is reasonable to suppose that the six amicronucleate individuals isolated to start the six amicronucleate lines were descended in the 'wild' state from a single animal, and likewise all the micronucleate animals were the progeny of

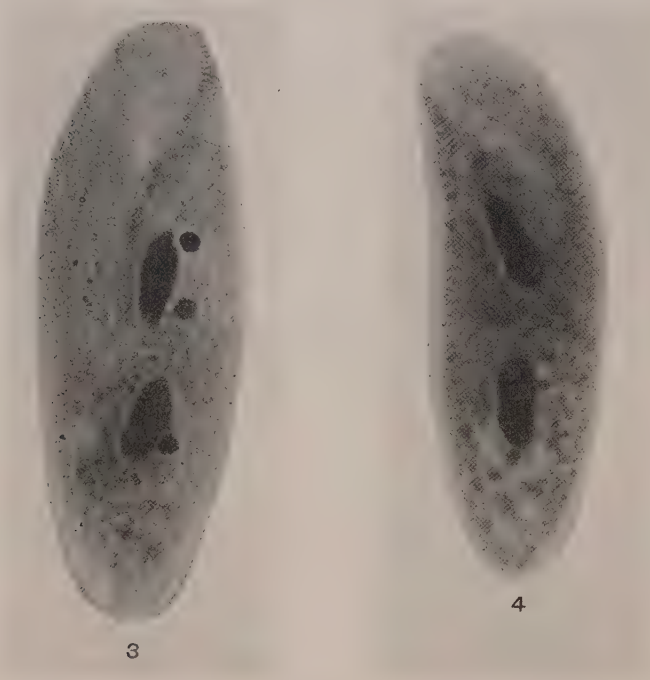

Fig. 3 Urostyla grandis. Micronucleate race, line Uc, 4th generation, May 14, 1920.

Fig. 4 Urostyla grandis. Amicronucleate race, line Ud, 8th generation, May 18, 1920.

a single animal. If so, then it was merely a matter of chance that four micronucleate and six amicronucleate lines were isolated from the 'wild' culture. At all events, it is highly interesting that the Urostyla population of the 'wild' culture exhibited two distinct racial types with respect to their nuclear apparatus.

PARAMECIUM CAUDATUM

During January, 1920, the author's course in protozoology made a study of the cytology of Paramecium aurelia and Paramecium caudatum and incidentally discovered a race which had the usual characteristics of P. caudatum except that no micronucleus could be demonstrated. It seems justifiable to conclude that a micronucleus was absent, though the race was not studied for a long period in pedigree culture. Apparently it was a race comparable to that recently discovered by Landis.

DISCUSSION

The facts in regard to amicronucleate races of ciliates are as yet too few to warrant an attempt to discuss from in a comprehensive way the questions which they present, though a brief survey and interpretation will serve to delineate the problem.

Confining attention to free-living ciliates, we find five species in which amicronucleate races have been definitely announced, viz., Oxytricha hymenostoma (Dawson), Oxytricha fallax (Woodruff), Urostyla grandis (Woodruff), Paramecium caudatum (Landis, Woodruff), and Didinium nasutum (Patten). To extend the survey to the general literature on the morphology of ciliates is not fruitful, because, unless the micronucleus was the special point of study, the evidence is of little value. Two records, however, may be mentioned which are highly significant. Thon, in 1905,[6] made a careful cytological study of Didinium, but was unable to discover a micronucleus, and Miss Moody, in 1912,[7] carried on a long pedigree culture of Spathidium spathula which revealed no micronuclei. It is difficult to conceive that either of these authors could have overlooked micronuclei if present. The micronuclei of Didinium, though small, are usually fairly easy to demonstrate when present. The micronuclei of Spathidium were clearly observed by Maupas,[8]

[6] K. Thon, Ueber den feineren Bau von Didinium nasutum. Archiv f. Protistenk., 1905, Bd. 5, S. 282.

[7] J. E. Moody, Observations on the life-history of two rare ciliates, Spathidium spathula and Actinobolus radians. Jour. Morph., 1912, vol. 23, p. 349.

[8] E. Maupas, Sur la multiplication des Infusoires ciliés. Arch. d. Zool. Expér. et Génér., 1888, 2e Série, T. 6, p. 247.

and are present in a pedigree race of this organism which the present writer now has under cultivation.[9] It is also significant that Miss Moody's race of Spathidium could not be induced to conjugate, whereas the writer's culture conjugates successfully at frequent intervals.[10]

The evidence at hand certainly shows that races of free-living ciliates exist which do not exhibit the orthodox nuclear apparatus. The evidence also indicates that amicronucleate forms are either unable to conjugate or, if cell fusion does occur, the result is abortive. It is certain that a morphologically defined micronucleus is not necessary for the continued existence by vegetative division of certain races of common free-living ciliates which typically have this cell organ.

If, on theoretical grounds, one is predisposed to believe that the presence of 'germinal' chromatin (ordinarily segregated in a micronucleus) is necessary for the life of the cell, then, since amicronucleate cells live and divide, the nucleus which is present must be regarded not as a macronucleus in sensu stricto, but as an amphinucleus. If this interpretation is correct, the relation between idio- and trophochromatin in the amphinucleus is apparently of such a nature that the former is unavailable for the conjugation phenomena, since the evidence to date indicates that conjugation either is not attempted or is abortive in amicronucleate races.

It is possible that amicronucleate races arise by the transformation of all the micronuclei, resulting from the reconstruction micronuclear divisions after conjugation or endomixis, into macronuclei. Such a result was observed by Prandtl in his study of the cytology of conjugation in Didinium, though he doubted if the animals with a nuclear heritage of this character were viable.[11]

[9] L. L. Woodruff and Hope Spencer, The structure and behavior of Spathidium spathula with special reference to the capture and ingestion of its prey. Jour. Exp. Zoöl. In press.

[10] L. L. Woodruff and Hope Spencer, The early effects of conjugation on the division rate of Spathidium. Proc. Soc. for Exp. Biol. and Med., 1921, vol. 18. The survival value of conjugation in the life history of Spathidium, Ibid., 1921, vol. 18.

[11] H. Prandtl, Die Konjugation von Didinium nasutum. Archiv f. Protistenk., 1906, Bd. 7, S. 251.

A concrete case will serve to make the point clear. In Paramecium caudatum, according to Calkins and Cull, the syncaryon divides three times to form eight micronuclei, four of which become transformed into macronuclei and four remain micronuclei. Since four micronuclei remain micronuclei, the differential which is at the basis of their persistence presumably was instistuted or became effective at the third micronuclear division—the division which segregated idiochromatin from trophochromatin. Calkins and Cull write:

> We have been unable to tell from morphological data which of the first eight somatic nuclei are destined to form four macronuclei; so far as we can tell there is no difference in the chromatin of these nuclei until that swelling begins which characterizes the young macronucleus. . . . . Here differentiation takes place, therefore, first by nuclear division and second by chromatin metamorphosis, and once transformed. . . . the macronucleus is ever after a macronucleus until its ultimate dissolution.[12]

Now a cell which received, in the normal course of reconstruction, such a micronucleus and macronucleus would be a typical animal—one in which the idiochromatin and trophochromatin was segregated into distinct bodies. Such an animal would represent the type which experimental work has shown to be, as a rule, non-viable in the absence of a micronucleus.

On the other hand, it is possible—perhaps probable when we recall the observations of Prandtl and of Patten—that under certain exigencies during cell reorganization the typical course of events may be disturbed so that the differential micronuclear division is suppressed or not precise and all the micronuclei metamorphose into 'macronuclei.' Such 'macronuclei' may be interpreted as amphinuclei. There is, a priori, no reason why nuclei of this character should not be adequate for all the typical life phenomena of the cell, except probably those involving endomixis or conjugation. Such apparently is the potentiality of the nuclear apparatus of the amicronucleate races thus far described.

[12] G. N. Calkins and S. W. Cull, The conjugation of Paramecium caudatum. Archiv f. Protistenk., 1907, Bd. 10, S. 406–407.

AUTHORS' ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 28

Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY, Vol. 33, No. 5, December, 1921

STUDIES ON THE RETINA

HISTOGENESIS OF THE VISUAL CELLS IN AMBLYSTOMA

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THIRTEEN FIGURES

INTRODUCTION

The anatomical features of the visual cells in larval Amblystoma have been described and figured by Laurens and Williams ('17). According to this account, the retina in larvae of 30 mm. possesses characteristic rods and cones in the approximate proportion of four to three. In their figure (p. 76) and in figure 11 in this paper the identity of these elements is clearly displayed. The rods are quite large with typical cylindrical outer segments and pear-shaped nuclei, the latter of which are considerably attenuated on their inner pole. The cones, of which there are three kinds, are much smaller elements characterized by the possession of conical outer segments, long myoids, and more or less oval nuclei. These nuclei are more deeply situated than are those of the rods, which typically comprise the outer cell layer of the external nuclear layer, and which project through the external limiting membrane for variable distances.

In looking over some sections of the retinae of young larvae, our attention was attracted to the striking similarity of the visual cells, all of which possessed conical outer segments and oval nuclei, characteristics typical of cones. The general impression was received that in early stages of development the visual cells are all of one type (cone-like), which later differentiate into the two distinct categories of elements which characterize the fully developed retina.

From our observations several developmental possibilities were suggested: first, if all of these typical cone-like elements are to be regarded as cones, then the rods, which do not appear in their distinctive form until later, represent transformation products of cones; secondly, that some of these elements functionally represent rods even though possessing anatomical features characteristic of cones; and, thirdly, that all of these cone-shaped elements represent indifferent visual cells and the characteristic rods and cones come about by divergent differentiation of a visual cell of low specialization.

In general, the rods are regarded as the more primitive type of visual cell (Graham Kerr, '19, and Parsons, '15) and the cones are regarded as specialized rods. If this be true, their appearance in ontogenetic development might be expected to antedate that of the cones—a condition just opposite to that which is suggested in the developing retina under consideration. The idea that cones represent specialized rods does not seem borne out by the condition found in many reptiles in which only cones are present (Detwiler, '16). If they do represent specialized rods, then the early developing visual cells in pure cone retinae should have to be regarded as rods—a condition which seems very unlikely.

According to Leboucq ('09), Magitot ('10) and Seefelder ('10), the two kinds of visual cells in the human retina develop simultaneously, and are mainly distinguishable by the fact that the axis of the diplosome (the centrosomes) is perpendicular to the long axis of the cone and parallel to the long axis of the rod. This is not entirely borne out in Seefelder's illustration which is reproduced in figure 12. The cones would also seem to be distinguishable by their much larger size. Cajal ('11) says that cones and rods evolve in essentially the same manner and it is difficult to distinguish them at the beginning (see also Fürst, '04).

Bernard ('03) and Cameron ('05 and '11), who studied the developing retina in amphibia, conclude that cones represent early stages in the formation of rods. Their conclusions are based on the fact that in early stages of the developing retina only conical elements are to be seen, while in later stages typically shaped rods predominate.

The difficulty in accepting the conclusions of Cameron and Bernard lies in the fact that the cone-like elements in the early stages, which they call cones, may functionally represent rods even though possessing the anatomical configuration characteristic of cones. The variability in the shape of the visual cells has made absolute rod and cone distinction difficult in some cases. We have already referred to this difficulty in an earlier paper (Laurens and Detwiler, '21, p. 225).

From our first survey of the developing retina in Amblystoma the conditions appeared to bear out the conclusions of Cameron and Bernard. However, more critical examination has shown that, although all the elements are cone-like in early stages of development, they possess characteristic features by which they can be divided into two groups, one of which can be readily identified as the progenitors of the definitive rods, the other of the cones. It seems hardly wise to regard the forerunners of the rods as functional cones even though possessing certain cone-like features.

METHODS

Our observations were made upon the retina of Amblystoma punctatum. The larvae were preserved in corrosive sublimate-acetic fixing fluid at successive twenty-four-hour intervals for a period of twenty-six days following the tail-bud stage of development. From this period on, specimens were fixed at successive intervals of from three to five days. Sections were cut 8μ in thickness and stained in Ehrlich's haematoxylin and erythrosin, iron haematoxylin and eosin, and in Held's molybdic acid haematoxylin.

OBSERVATIONS

The visual elements make their initial appearance in retinae of approximately eleven days after the tail-bud stage of development.[1] They are first seen in the posterior portion of the optic cup, just above the point of entrance of the optic nerve—a region corresponding to the optical axis of the eye (fig. 1). Prior to this

[1]All references to ages are based upon days after the tail-bud stage of development.

period, the nuclei of the external nuclear layer show numerous mitoses.

The first step in the formation of the visual cells is the simultaneous appearance of a protoplasmic bud and a small achromatic

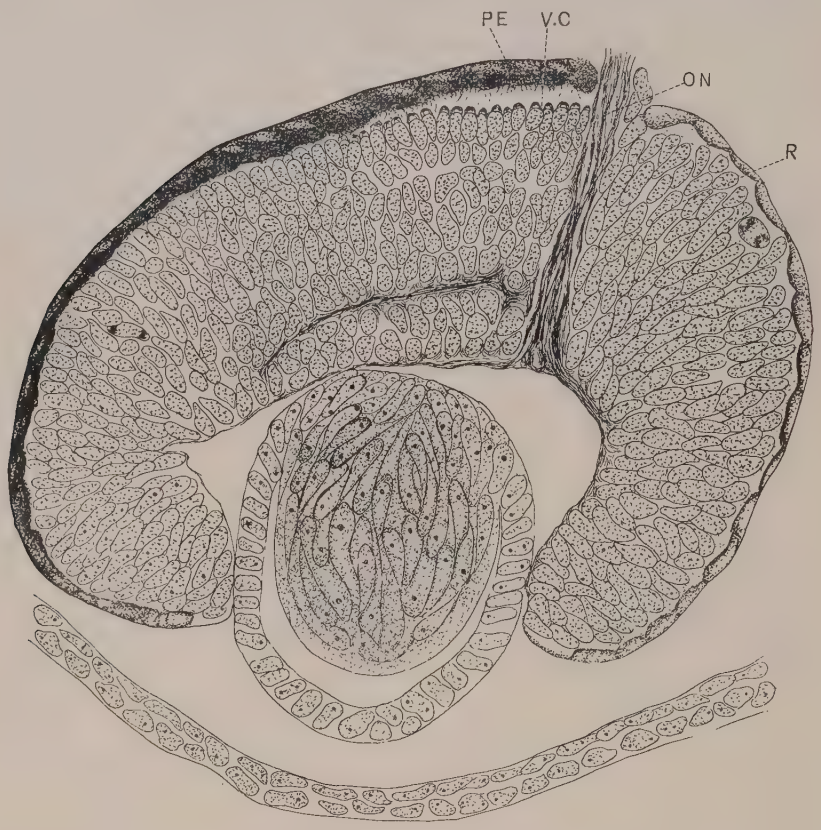


Fig. 1 Transverse section of optic cup and lens in an embryo of 11 days after the tail-bud stage of development. *ON*, optic nerve; *PE*, pigment epithelium; *R*, retina; *V.C*, developing visual cells. × 275.

globule on the pole of the nucleus applied to the external limiting membrane (fig. 2, A and B). In sections stained with iron haematoxylin, groups of deeply stained granules are usually seen just distal to the globule. The protoplasmic buds appear to be pushed through the external limiting membrane.

The conditions in retinae of forty-eight hours further development are seen in figure 3. The protoplasmic buds have become larger and assume a blunt conical shape. The globules occupy the proximal portion of the protoplasmic mass, whereas distal to them occur masses of deeply staining granules similar to the conditions shown in figure 2.

As regards the origin of the clear achromatic globule, Cameron claims that it is extruded from the nucleus. He supports his contention by observations which both he and Bernard made in

Fig. 2 Developing visual cells from the retina in an embryo of 11 days after the tail-bud stage of development. × 1040.

Fig. 3 Developing visual cells from the retina of an embryo of 13 days after the tail-bud stage. × 1040.

which they found a collapsed condition of the nucleus subsequent to the supposed 'sudden' discharge of its achromatic material. This view is contradicted by Leplat ('13). Graham Kerr ('19) states that the 'fatty' globule, which stains black with osmic acid, has its origin in the protoplasmic bud from the primitive visual cells.

Cameron attributes to this clear globule the power of ingesting pigment from the hexagonal pigment cells, and the intensely stained granules which he found on the summit of this globule (figs. 2 and 3) he claims to be the pigment ingested from the pig-

ment layer. Because of the fact that only the merest trace of cytoplasm can be shown to envelop the nucleus of the primitive visual cell, Cameron claims that the visual element cannot be of cytoplasmic origin, and he thus believes that it is compositely developed from the nucleus and from the ingested pigment of the epithelial layer.

It seems to us that, even if pigment ingestion can be really observed, the fact must not be overlooked that pigment ingestion could not take place unless there were cytoplasm present to ingest it. Cameron holds that the clear globule does the ingesting, but it so happens that the heavily stained granules which he saw, as well as those which we have observed, do not lie within the globule, but in the cytoplasm beyond it (fig. 3). Cameron himself remarks, " . . . . and the product of the ingestion of the pigment becomes deposited upon the summit of the globule in the form of a substance which stains intensely with iron-alum-haematoxylin. In this manner, layer after layer becomes superimposed until all the visual elements without exception now assume the shape of cones, with the achromatic globules, much reduced in size, situated in their bases."

From our observations the origin of the globule cannot be stated decisively, but whenever it is present, it is always surrounded by cytoplasm and the two seem to appear simultaneously. In many initial developing cases, cytoplasmic buds can be seen in which there is no globule and yet they contain the heavy granules which are shown in figures 2 and 3. That these granules should represent products of epithelial pigment seems very doubtful from our observations. If they are ingested under the chemotactic influence of the globule, as Cameron says, then visual cell buds which lack the globule should be devoid of this deeply staining substance. Moreover, the pigment needles of the hexagonal cells are not stained nearly so heavily as are those in the visual cell buds. The former are brownish and only appear black when heavily massed. The granules in the visual cell bud are jet black in our preparations, even when appearing singly. Our observations suggest that the heavily stained granules are elaborated in the cytoplasm of the visual cell and that the globule

comes about through partial vacuolization of the cytoplasm. Occasionally, small refractive granules can be seen in the clear globule. These may represent ingested pigment, but the heavily stained ones situated distal to the globule and which comprise a large part of the visual cell, certainly give no indication of originating from the pigment epithelium.

Leplat ('13) has investigated the rôle of mitochondria in connection with the development of the rods and cones in the chick. He also found deeply stained granules in the initial protoplasmic buds. These granules, which he has called mitochondria, are to be seen in figure 13A, which is a reproduction of one of his illustrations. They are seen to bear a striking resemblance to those which we have observed (figs. 2 and 3), yet our preparations were fixed with the aid of 5 per cent acetic acid, which is a mitochondrial solvent. Leplat regards the transverse striation of the external segment and its cleavage into discs (fig, 13 B), as the expression of its mode of construction from mitochondria. In our preparations a similar transverse arrangement of the material is readily seen (fig. 5), though they cannot be regarded as mitochondrial granules for reasons previously stated. They may, however, represent chemical transformation products of mitochondria with the retention of the plan of the mitochondrial arrangement.

No evidence of rod and cone identity based on the posture of the diplosome could be gathered from our preparations. In iron-haematoxylin stained sections, the protoplasmic buds exhibited only large groups of granules as seen in figures 2 and 3. If a typical diplosome is present, such as is shown in Seefelder's illustration of the developing human retina (fig. 12) and Lebоucq ('09) and Fürst ('04), it is entirely masked by other granules.

Lebоucq and Seefelder describe the cone bud as being much stouter than that of the rod (fig. 12). This figure also shows that the nuclei of the cones lie in a plane closer to the external limiting membrane than the rod nuclei. In Amblystoma the initial protoplasmic buds are approximately of the same size. The visual elements in a retina of several days' further development show marked differences in size (fig. 4). This relationship remains

unchanged, and when the retina is fully differentiated the rod nuclei are seen to constitute the outer row of the external nuclear layer. The same relationship between the position of the rod and cone nuclei was found in the eyes of the alligator (Laurens and Detwiler, '21). In the human retina the cone nuclei occupy the more superficial position.

In retinae of fifteen days the visual cells typically show two groups of granules (fig. 4), between which can be seen a clear re-

4

5

Fig. 4 Developing visual cells from the retina of a larva of 15 days following the tail-bud stage of development. The larger elements are the progenitors of the rods; the smaller, of the cones. × 1040.

Fig. 5 Visual cells from the retina of a larva of 17 days following the tail-bud stage of development. The three large conical-shaped elements are differentiating rods. Their nuclei lie partly beyond the external limiting membrane. The granular material of their outer segments shows a lamellar arrangement characteristic of the fully differentiated rod (fig. 11). Arrow indicates double cone. × 1040.

fractive disc marking the position of the oil drop. The original clear globule is retained in the proximal portion of the visual element and apparently develops the paraboloid. The proximal group of granules develops into the ellipsoid portion of the inner segment. The distal group of granules, as far as can be ascertained, becomes transformed into the typical granular discs which characterize the outer segment particularly in the rod. How this group of granules develops is uncertain from our preparations. That they may be separated off from the original

group by liquefaction is suggested by observations which show that in the center of the single group of granules a clear area (granule-free) can be seen as shown in figure 3. Whether or not this is actually the case, cannot be definitely determined. At any rate, the clear refractive oil drop or disc which separates these two groups of granules gives no indication of coming from the original globule, which persists in the proximal portion of the inner segment as the paraboloid.

Double cones can be seen in early stages of development, as illustrated in figure 5. These double elements give no indications as having arisen by division of single elements. When they are first observed, each member possesses its own nucleus. The total absence of mitotic figures at this stage when double cones first make their appearance suggests that they result from the fusion of preexisting single elements rather than by division.

In the retina of seventeen days (fig. 5), the inner and outer segments of the visual cells are readily distinguishable. The inner group of granules has enlarged and is more deeply stained. They constitute the ellipsoid portion of the inner segment. The granular material of the outer segment of the large conical visual cells has undergone cleavage into discs which are separated by clear non-stainable bands. The lamellar arrangement of this granular material is quite prominent in the outer segment of the large visual cells, but less so in the smaller ones (fig. 5). The difference in the structural arrangement of the material in the outer segment which can be observed at this period serves as another index that the individuality of the larger conical-shaped elements is different from that of the smaller ones. Although these large elements are conical in shape, the structural arrangement of the material in the outer segment is quite similar to that which characterizes the fully differentiated rod, and all the evidence suggests that these large elements are not to be regarded as cones.

In embryos of twenty-one days (fig. 6) the visual cells show increased growth. The nuclei of the large conical-shaped elements (differentiating rods) begin to assume a pear-shape—a condition which, added to their more external situation, readily

distinguishes them from the cone nuclei. The latter are oval in shape and occupy a deeper level.

A count of the two kinds of visual cells shows that the number of larger elements slightly exceeds that of the smaller. The ratio is approximately the same as the rod-cone ratio in the fully differentiated retina, viz., four to three.

Successive stages in the development of the retinal visual cells are shown in figures 7, 8, 9, 10, and 11. These figures show the developmental conditions at twenty-six, thirty-one, thirty-six,

6

Fig. 6 Visual cells from the retina of an embryo of 21 days after the tail-bud stage of development. The nuclei of the differentiating rods are being drawn out on their inner pole, (cf. fig. 11). The cone nuclei are more deeply situated and are oval in shape. × 1040.

forty, and fifty days, respectively. The difference in size between the two types of cells gradually increases as development proceeds. The large conical element gradually becomes more and more rod-shaped, and the final typical rod form which it acquires is illustrated in figure 11. Throughout these successive stages, the nucleus of this visual cell (rod) not only becomes more and more drawn out on the inner pole, but it gradually increases in its stainability, as is shown in figures 10 and 11. These nuclei extend for variable distances beyond the external limiting membrane, whereas the cone nuclei lie beneath it. The outer segment of this element shows throughout a disc-like arrangement of its granular material.

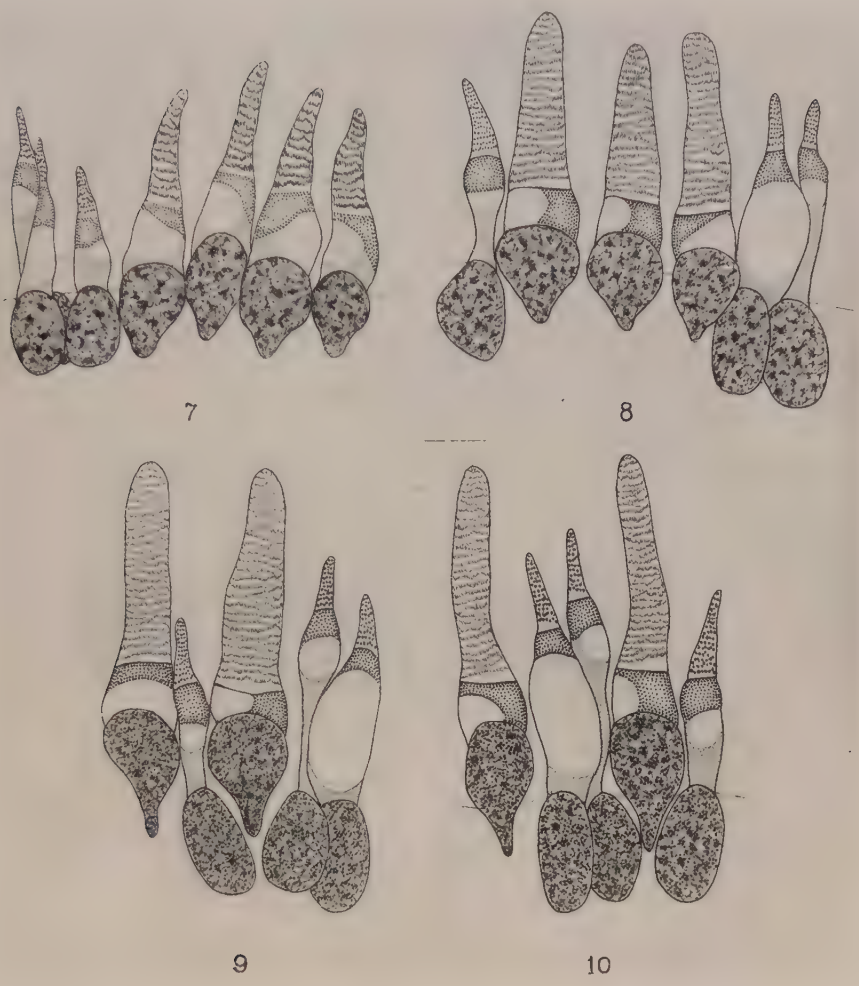


Fig. 7 Double cone, single cone, and four rods from the retina of a larva of 26 days following tail-bud stage. × 1040.

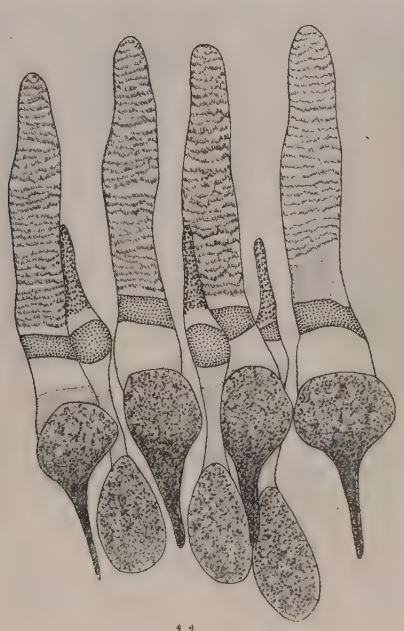
Fig. 8 Double cone, single cone, and three rods from the retina of a larva of 31 days after tail-bud stage. × 1040.

Fig. 9 Double cone, single cone, and two rods from the retina of a larva of 36 days following tail-bud stage of development. × 1040.

Fig. 10 Double cone, single cone and two rods from the retina of a larva of 40 days following tail-bud stage of development. × 1040.

In the fully formed rod the myoid is exceedingly short. The presence of a narrow refractive disc between the outer and inner segments is demonstrable in most of the rods. In others it appears to be lacking.

Owing to the greater growth of the rods as development proceeds, the difference in size between the cones and the rods in the fully differentiated retina (fig. 11) is greater than in earlier



11

Fig. 11 Four rods and three single cones from retina of larva of 50 days after tail-bud stage. × 1040.

stages of development (fig. 8). The final form of the cones is seen in figure 11. Their shape and structure agree with the description given by Laurens and Williams ('17). The outer segments are bluntly conical, and the granular material is not so characteristically arranged in lamellae as in the rods. As regards the inner segment, the size of the paraboloid is variable and is present in single as well as in both members of the double cones.

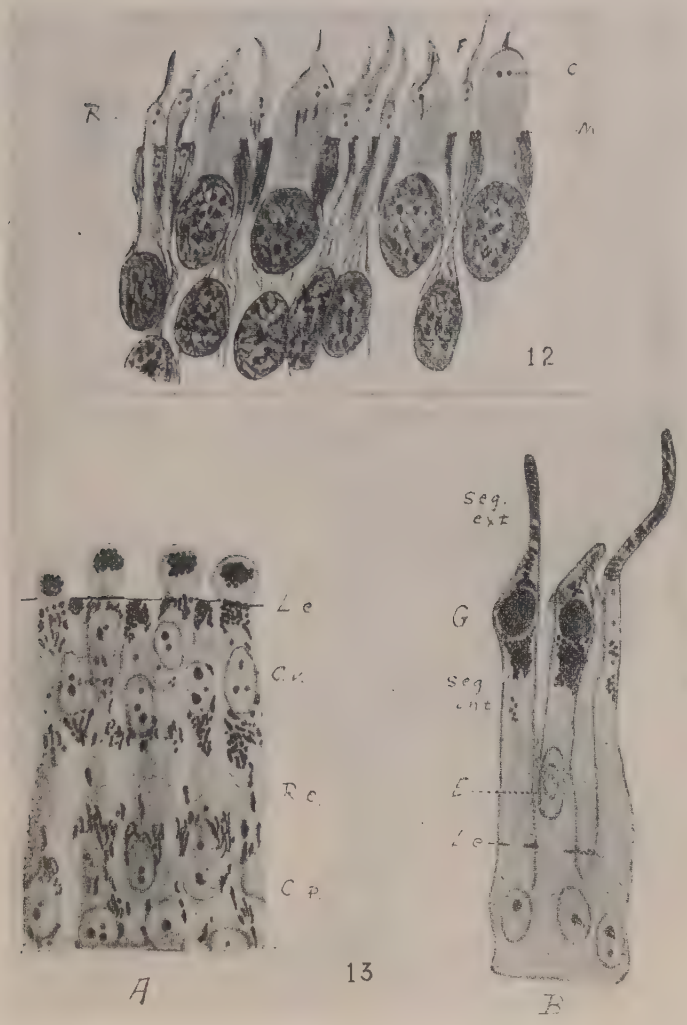


Fig. 12 Developing rod and cone visual cells, from the retina of a 345-mm. (six months) human fetus. *M*, diplosome in a Müller's fiber at the level of the external limiting membrane; *C*, diplosome in a cone cell; *F*, fiber growing out from the cone diplosome; *R*, diplosome in a rod visual cell. × 1000. (After Seefelder. From Jordan and Ferguson's Text-book of Histology.)

Fig. 13 Two early stages in the development of the rod and cone visual cells in the chick. A, from twelve-day embryo, showing the visual cell-buds containing mitochondria. *L.e.*, external limiting membrane; *C. v.*, visual cell; *R.e.*, external reticular layer; *C.p.*, bipolar cells. B, from one-day-old chick, showing two complete rods and one cone, both elements containing a large lipoid spherule (*G*) and mitochondria. The cone contains an ellipsoid (*E*); *seg. int.*, internal segment; *seg. ext.*, external segment. × 2000. (After Leplat. From Jordan and Ferguson's Text-book of Histology.)

The character of the connections of the visual cells with the internal nuclear layer could not be determined. In preparations stained with Held's molybdic acid haematoxylin, the internal prolongation of the rod nuclei could be followed for a considerable distance, but its final connection could not be seen.

With the establishment of the character of the centripetal connections of the visual cells which remains to be brought out by impregnation or other methods, in addition to observations on the functional behavior prior to the period when typical rods first appear, more light may be thrown upon the identity of the developing visual cells in Amphibia.

SUMMARY

1. The fully differentiated retina of Amblystoma punctatum possesses characteristic rods and cones (fig. 11).

2. The initial stage in the development of the visual cell is the production of a protoplasmic bud and a clear achromatic globule from the cells of the external nuclear layer (fig. 2). The globule, which becomes the paraboloid of the inner segment, appears to be of cytoplasmic origin, and not of nuclear, as asserted by Cameron.

3. In early stages of the development of the visual cells (eleven to thirteen days after tail-bud stage) masses of deeply staining granules are seen in iron-haematoxylin preparations (figs. 2 and 3). These granules contribute mainly to the formation of the ellipsoid of the inner segment and to the granular material of the outer segment. There is no evidence from our preparations which indicates that they are products of pigment ingested from the epithelial layer. They may represent transformation products of mitochondria.

4. In these early stages the visual cells are all cone-like (figs. 2 and 3), agreeing in this respect with the conditions characteristic of the developing amphibian retina (Cameron and Bernard). Rod and cone identity based on the position of the diplosome such as has been described by Fürst, Leboucq, and Seefelder has not been demonstrated. Rods and cones are later differentiated from the primitive, non-specialized visual cells.

5. Rods do not appear in their definitive form until relatively late in development. Their progenitors consist of large conical-shaped elements which possess, in early stages of development (fifteen days), an individuality by which they can be distinguished from the true cones. These elements are larger, their nuclei occupy a more external position than that of the cones, and the granular material of their outer segments becomes arranged in lamellae characteristic of the fully differentiated rod (figs. 5 and 6). The ratio of these conical-shaped rod progenitors to that of the cones is approximately the same as that of the rod-cone ratio in the fully differentiated retina (viz., four to three).

6. The two kinds of conical-shaped visual elements as seen in figure 4 (large and small) are regarded as visual cells of low specialization, which develop, respectively, into characteristic rods and cones by divergent differentiation (fig. 11). This interpretation is not in support of the view advanced by Cameron, who regards all of the conical-shaped visual cells in early stages of amphibian development as cones and the later appearing rods as transformed cones.

7. Double cones appear very early in development (fig. 5). The absence of mitotic figures in the nuclei of the external nuclear layer during the period when double cones first appear suggests that they are formed from the fusion of single elements rather than from division.

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